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Water-In-Oil Emulsifying Agents. III. Studies of Emulsifying Efficiencies*

By E. L. Cataline, Frank Reinish† and S. F. Jeffries‡

Although wool fat has been used as an emulsifying agent for many years, it is only recently that investigation has indicated that substances other than cholesterol and related alcohols may be responsible for the ability of wool fat to promote the emulsification of rather sizable quantities of water in oily materials. Powers and his co-workers (1) were able to show that, while cholesteryl esters of fatty acids when used alone are not capable of promoting emulsification, they markedly increase the emulsifying efficiency of solutions of cholesterol in white petrolatum. More recently, Johnston and Lee (2) have applied this information to the problem of ointment base formulation.

The rather remarkable ability of some cholesteryl esters to enhance the emuslifying efficiency of cholesterol-white petrolatum mixtures more than threefold is as yet unexplained and this series of investigations was undertaken in an attempt to gain information which might clarify the problem. A second purpose was to develop, if possible, a readily available, inexpensive, stable water-absorbent ointment base devoid of the objectionable features of lanolin-containing absorption bases. Studies of a number of experimental bases will be reported in future communications.

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EXPERIMENTAL

Preparation of the Test Sample.—In all but three instances the components of the mixture dissolved when the appropriate quantities were heated with white petrolatum at 125–135° C. for ten minutes.

When the mixture of cholesterol and adipic acid was prepared by this method, dark, oily globules, insoluble in the white petrolatum, were formed. A homogeneous solution was obtained when the petrolatum was heated in an oil bath to 175–180° C., the adipic acid added, the mixture maintained at the above temperature until the acid had dissolved, and the cholesterol then added in small portions.

For the production of smooth suspensions of acids which did not dissolve in white petrolatum the following procedure was found desirable: The cholesterol was dissolved in the white petrolatum by heating at 125–135° C., the mixture allowed to cool and the finely powdered acid incorporated into the base by mixing on an ointment slab. The mixture was remelted on the water bath and was then stirred constantly while cooling.

Determination of Emulsifying Efficiency.—The following procedure is a modification of that described by Powers, Leask and Warner (1).

The apparatus consisted of a spiral glass stirrer driven by an electric motor through a pulley system so arranged that the stirrer rotated at about 2200 r. p. m. Emulsification was effected in ordinary 8 x 1-in. test tubes which were held in place vertically

by two adjustable clamps. When a tube was in place the stirrer extended to within about 1/4 in. of the bottom. By releasing the tension on the clamps, the tube could be lowered and raised in relation to the stirrer, thus assuring uniform mixing.

The base, prepared as indicated above, was melted and a standard quantity of 5 Gm. poured into each of a series of test tubes. The tubes were kept in a water bath maintained at 60–70° C. until immediately before the determination.

Before each test run the stirrer was warmed by immersing it in a test tube containing distilled water heated to 60–70° C. When the stirrer was not warmed the results were usually erratic. While the stirrer was being warmed, one of the series of charged tubes was removed from the water bath and 2.5 cc. of distilled water, heated to 60–70°C., was added from a graduated pipette. The tube containing the water used to warm the stirrer was removed, the stirrer quickly wiped dry and the charged tube was immediately clamped into position. The motor was started and as soon as emulsification had commenced the tube was slowly lowered and raised several times. Stirring was continued until the temperature of the tube had fallen to approximately 30–35° C.

Under identical conditions water was added, in increasing amounts, to the other tubes of the series, the increment being 2.5 cc. in each instance. The series was terminated with the first tube in which an appreciable portion of the water was not emulsified. All determinations were run at least in duplicate, usually by different operators.

Emulsification usually commenced shortly after stirring was begun and proceeded uniformly until either all or the maximum volume of water was emulsified. It was observed that when the temperature of the mixture was initially above 70° C. emulsification was usually somewhat delayed. In those instances in which the water was completely emulsified the emulsion was deposited around the periphery of the tube as a thick layer which ascended the wall of the tube to a distance which was roughly proportional to the volume of water emulsified. When an excess of water was present the emulsion rotated freely in the tube.

The tubes containing the emulsions were tightly corked and stored in the dark at room temperature for two months. The emulsions were then examined and a decision reached as to the maximum amount of water emulsified by the standard quantity of base.

DISCUSSION

The esters employed in this investigation were those described in the second paper of this series (3).

The emulsifying efficiencies of the various mixtures of alcohols and esters studied are summarized in Table I. Since a 3% solution of cholesterol in white petrolatum possesses an emulsifying efficiency of about 250%, it is apparent that in all instances studied the emulsifying efficiency of the cholesterol-white petrolatum mixture is increased by the addition of cholesteryl and cetyl esters. Cholesteryl esters of the saturated, straight-chain, monocarboxylic acids up to lauric acid doubled the efficiency of the cholesterol-white petrolatum mixture, cholesteryl laurate trebled the efficiency while the higher esters brought about still greater increases.

It was thought that the increase in efficiency might be related to increase in molecular weight. If this were so it would appear that the normal esters of saturated, straight-chain dicarboxylic acids would, in consequence of their greater molecular weight, bring about still greater increases in emulsifying efficiency than those produced by the esters of the monocarboxylic acids. Comparison of the effects of esters of acids of the two types having the same number of carbon atoms indicates that this may be true to a limited extent. Dicholesteryl oxalate increased the efficiency of cholesterol to 600% whereas cholesteryl acetate brought about an increase to only 500%. The same type of difference is seen when the effects of dicholesteryl succinate and dicholesteryl adipate are compared with those of cholesteryl *n*-butyrate and cholesteryl *n*-caproate, respectively. However, none of the cholesteryl esters studied increased the efficiency of the white petrolatum solution of cholesterol so much as did cholesteryl stearate although their molecular weights are all considerably greater than that of the stearate.

Although the cetyl esters did increase the efficiency of the cholesterol-white petrolatum mixture, in only one instance (dicetyl adipate) did they appear to be as satisfactory as the corresponding cholesteryl esters. Mixtures of cetyl esters with cetyl alcohol exhibited virtually no emulsifying power. The white petrolatum used in these studies possessed an emulsifying efficiency of about 50% and upon the addition of cetyl ester-cetyl alcohol mixtures there was no increase. Likewise, it was shown in a separate experi-

TABLE I.—EMULSIFYING EFFICIENCIES OF MIXTURES IN WHITE PETROLATUM

Acid	Emulsifying Efficiency, % — Mixture I[a]	II[a]	III[a]	IV[a]
Acetic	500	...	..	...
Oxalic (di-ester)	600	550	50	425
n-Butyric	500	...	..	...
Succinic (di-ester)	650	600	50	450
n-Caproic	500	...	..	...
Adipic (di-ester)	600	600	50	475
Lauric	600	550	50	400
Myristic	700	500	50	350
Palmitic	700	550	50	375
Stearic	800	600	50	500
12-Hydroxystearic	850[b]	800	50	450

[a] Mixture I = Cholesterol, 3%, Cholesterol Ester, 3%. Mixture II = Cholesterol, 3%, Cetyl Ester, 3%. Mixture III = Cetyl Alcohol, 3%, Cetyl Ester, 3%. Mixture IV = Cholesterol, 4.5%, Acid, 1.5%.

[b] The cholesteryl 12-hydroxystearate employed was not pure (Ref. 3, p. 108).

ment that cetyl alcohol alone in 3% concentration was incapable of increasing the efficiency of the white petrolatum. In fact, the addition of 3% cetyl alcohol to a solution of 3% of cholesterol and 3% cholesteryl stearate in white petrolatum brought about a decrease in efficiency from 800% to 750% and when 3% cetyl stearate was added to the cetyl alcohol-cholesterol-cholesteryl stearate mixture the efficiency fell to about 400%. It would seem, therefore, that mixtures of cetyl alcohol with cetyl esters are not satisfactory for use where large concentrations of water are to be emulsified in white petrolatum by means of cholesterol-cholesteryl ester mixtures.

According to the orientation theory of emulsification (4) compounds possessing both an oil-soluble, non-polar group and a polar, water-soluble group should be more efficient emulsifying agents than those which are solely oil-soluble. Although the evidence is meager, it appears that the introduction of a hydroxyl group into esters of the type under discussion may bring about an increased efficiency. Both cetyl 12-hydroxystearate and the impure cholesteryl 12-hydroxystearate which we have obtained proved more efficient than the corresponding stearates. It is hoped that this matter may be investigated further.

In order to determine whether esterification of the acids involved as esters in this study was necessary to bring about large increases in emulsifying efficiency, a number of mixtures containing 4.5% cholesterol and 1.5% of the acid were studied by the procedure described above. The percentages mentioned were selected because they approximated the relative proportions of cholesterol and acid in the mixtures containing 3% cholesterol and 3% cholesteryl ester. The results are recorded in the last column of Table I. It is apparent that in no instance did the emulsifying efficiency of a cholesterol-acid mixture approach that of the corresponding cholesterol-ester mixture although all cholesterol-acid mixtures were more efficient than cholesterol alone in white petrolatum.

SUMMARY AND CONCLUSIONS

1. The effect of various mixtures of cholesterol, cholesterol esters, cetyl alcohol and cetyl esters on the emulsifying efficiency of solutions of cholesterol or cetyl alcohol in white petrolatum has been studied.

2. Straight-chain, saturated monocarboxylic acid esters of cholesterol increase the emulsifying efficiency of cholesterol-white petrolatum mixtures as much as threefold. The increase in efficiency is roughly proportional to increase in molecular weight when the latter is relatively high.

3. Normal cholesteryl esters of saturated, straight-chain dicarboxylic acids appear to increase the emulsifying efficiency of cholesterol-white petrolatum mixtures more than do the corresponding esters of monocarboxylic acids. However, in no instance studied did this increase in emulsifying efficiency equal that brought about by cholesteryl stearate.

4. Esters of cetyl alcohol are capable of increasing the emulsifying efficiency of solutions of cholesterol in white petrolatum but mixtures of cetyl alcohol and cetyl esters exhibited little emulsifying ability.

5. In the two instances studied, the presence of a free hydroxyl group in the ester appeared to impart greater emulsifying efficiency.

6. Mixtures of cholesterol and free acids were less efficient emulsifiers than mixtures of cholesterol and the cholesteryl esters of the acids.

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Water-In-Oil Emulsifying Agents. IV. Further Studies of Emulsifying Efficiencies. Stability of Some Emulsions*

By E. L. Cataline, Frank Reinish† and Lee Worrell

One of the more serious criticisms of the emulsions prepared from the cholesterol-cholesteryl ester absorption bases so far described is that they are not satisfactory in so far as stability is concerned. The number of types of medication which may be incorporated in them is limited since many medicaments either break or reverse the phases of the emulsion systems produced when the base is mixed with water. Upon standing for varying lengths of time many of the emulsions separate even though the medication may not be involved in the separation. Furthermore, when the emulsions are rubbed on the skin they usually separate, the aqueous phase appears in the form of droplets and the appearance and "feel" of the ointments are definitely unsatisfactory. The studies described below were undertaken in an attempt to overcome some of these difficulties.

EXPERIMENTAL

Determination of Emulsifying Efficiency.—The preparation of the test samples and absorption bases and the determination of emulsifying efficiencies were carried out as previously described (1).

Determination of Stability of Experimental Absorption Bases

(a) Shelf Tests.—All of the emulsions for which stability data are reported were stored in indirect light in the laboratory and observed from time to time. In the accompanying tables, a plus sign indicates that the emulsion was stable for at least two months.

(b) Estimation of Water-Retaining Properties of Emulsions when Rubbed upon the Skin (the "Skin Test").—The procedure described below was devised so as to make it possible to evaluate, relatively, the water-retaining properties of the emulsions when rubbed upon the skin.

A control was prepared by emulsifying 50 Gm. of distilled water in 10 Gm. of a solution of 3% each of cholesterol and cholesteryl stearate in white petrolatum. A small quantity of this control emulsion was applied to and spread upon the dorsal surface of the hand with more or less uniform strokes of the index finger of the other hand. During the rubbing the behavior of the control in relation to its water retention was noted. A similar quantity of the experimental emulsion was then applied in a like manner to another portion of the dorsal surface of the hand and its behavior compared with that of the control. In the tables below, a plus sign indicates that the experimental emulsion seemed to retain the emulsified water more satisfactorily than did the control, while a minus sign indicates less satisfactory behavior. Borderline cases are indicated by a plus-minus sign. In the few instances in which the behavior of the experimental emulsions was greatly superior to that of the control, the skin test stability was recorded as "two plus." In no instance did the emulsion retain the water completely. While this test is obviously subject to the variations inherent in any organoleptic test, it is interesting to note that results obtained by several individuals varied to an exceedingly small degree.

* Received August 7, 1944, from the University of Michigan College of Pharmacy, Ann Arbor, Mich.

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DISCUSSION

Emulsifying Efficiencies.—Since it has been shown (1, 2, 3) that the addition of 3% of cholesteryl stearate to a solution containing 3% of cholesterol in white petrolatum brings about an increase in emulsifying efficiency from 250% for the cholesterol solution to 800% in the case of the cholesterol-cholesteryl stearate-white petrolatum mixture, it seemed desirable to determine the effect of the addition of other cholesteryl esters to the latter mixture. The data in Table I

TABLE I.—EMULSIFYING EFFICIENCIES OF MIXTURES OF CHOLESTEROL (3%), CHOLESTERYL STEARATE (3%) AND CHOLESTERYL ESTER (3%)

No.	Cholesteryl Ester	Emulsifying Efficiency, %
1	Acetate	700
2	Butyrate	500
3	Caproate	550
4	Laurate	700
5	Myristate	725
6	Palmitate	800

indicate that the addition of fatty acid esters below the palmitate is accompanied by a decrease in the emulsifying efficiency while the palmitate does not detract from the effect of the cholesteryl stearate. The anomalous behavior of the acetate is difficult to understand since a mixture of cholesterol and cholesteryl acetate in white petrolatum has an emulsifying efficiency of only 500%. All these emulsions were stable to shelf testing for a period of at least two months and most of them had not separated after ten months' storage.

Previous investigators (2, 3) agree that in the formulation of ointment bases from cholesterol and cholesteryl stearate, the concentration of each in white petrolatum need not exceed 3%. To the present, however,

there are no experimental data to indicate whether or not the concentration of either may be reduced without materially reducing the emulsifying efficiency of the mixture. The data in Table II indicate that a reduction in the concentration of either substance is accompanied by a decrease in emulsifying efficiency and that reduction of the concentration of cholesterol is accompanied by a relatively greater decrease in efficiency than is a proportional reduction in the concentration of the ester. This result is to be expected when it is remembered that cholesteryl esters, when used alone, have no emulsifying action (2).

TABLE II.—EMULSIFYING EFFICIENCIES OF MIXTURES OF CHOLESTEROL AND CHOLESTERYL STEARATE

No.	Cholesterol, %	Cholesteryl Stearate, %	Emulsifying Efficiency, %
7	3.0	3.0	800
8	3.0	2.0	675
9	3.0	1.0	550
10	3.0	0.5	400
11	2.0	3.0	475
12	1.0	3.0	250
13	0.5	3.0	200

It was thought that glycerin might have a stabilizing effect upon the emulsions under consideration and it was desirable to determine its effect upon the emulsifying efficiency of the cholesterol-cholesteryl stearate mixture. Table III indicates that the addition of glycerin in increasing amounts leads to rather large reduction in emulsifying efficiency. Shelf test stability was good but the skin test quality was unsatisfactory. The use of glycerin in amounts larger than 1% would seem inadvisable where large quantities of water are to be emulsified in white petrolatum by cholesterol-cholesteryl ester mixtures.

TABLE III.—EMULSIFYING EFFICIENCIES OF MIXTURES OF CHOLESTEROL (3%), CHOLESTERYL STEARATE (3%) AND GLYCERIN

No.	Glycerin, %	Emulsifying Efficiency, %
14	0.5	750
15	1.0	650
16	1.5	450
17	2.0	300

While soybean lecithin is stated to promote oil-in-water emulsions (4, 5), and it might be suspected that it would exhibit an action antagonistic to that of the emulsifying agents studied in these investigations, the data in Table IV show that soybean lecithin, when used in amounts up to and including 2%, definitely enhanced the emulsifying efficiency of a mixture of cholesterol and cholesteryl stearate. In addition, the lecithin increased the speed of emulsification and the emulsions seemed more viscous than that produced by the cholesterol-ester mixture alone. However, the emulsions were not stable, water separation occurring after only two or three days.

TABLE IV.—EMULSIFYING EFFICIENCIES OF MIXTURES OF CHOLESTEROL (3%), CHOLESTERYL STEARATE (3%) AND SOYBEAN LECITHIN

No.	Soybean Lecithin, %	Emulsifying Efficiency, %
18	0.5	850
19	1.0	950
20	2.0	950

TABLE V—STABILITY OF EMULSIONS[a]

No.	Lecithin, %	Cetyl Alcohol, %	Other Substances	Shelf Test	Skin Test
21	1	...	....	+	+
22	1	1	....	+	++
23	1	5	....	+	++
24	...	...	Cetyl 12-hydroxystearate (3%)	−	+[b]
25	1	...	....	−	+[b]
26	...	...	Sorbitol (2%)	+	+
27	1	...	Sorbitol (2%)	[c]	[d]
28	1	1	Sorbitol (2%)	[c]	[d]
29	1	2	Sorbitol (2%)	[c]	[d]
30	...	...	Cocoa butter (2%)	−	+[b]
31	1	...	Cocoa butter (1%)	−	+[b]
32	1	1	Cocoa butter (1%)	+	±
33	...	...	Glyceryl monostearate (1%)	[e]	[e]
34	...	...	Glyceryl monoricinoleate (2%)	[e]	[e]
35	1	...	Diglycol stearate (1%)	+	±
36	1	1	Castor oil (2%)	−	±[b]
37	1	...	Hydrogenated castor oil (1%)	−	±[b]
38	1	1	Heavy mineral oil (2%)	+	±
39	...	...	Carbitol (2%)	−	±[b]
40	...	...	Triacetin (2%)	−	±[b]

[a] The emulsions were prepared by incorporating 50 Gm. of distilled water into 10 Gm. of a base consisting of 3% cholesterol, 3% cholesteryl stearate and the indicated amount of the above substances in white petrolatum.
[b] Skin test quality determined before separation.
[c] Gradually softened at room temperature.
[d] Liquefied when applied to the skin.
[e] Did not emulsify.

Stability of Emulsions.—As mentioned above, one of the serious problems in formulating emulsified ointments of the water-in-oil type is the instability of the preparations, especially when rubbed upon the skin. Johnston and Lee (3) found that combinations of cholesterol, cholesteryl esters, higher alcohols, and waxes failed to prove satisfactory but that the addition of 25% of anhydrous lanolin to a cholesterol-cholesteryl stearate-white petrolatum mixture gave, upon the addition of water, an emulsion which "showed no separation of water when rubbed upon the skin. Its spreading qualities were quite satisfactory, in fact just as good as the emulsions made

from 2 of the much-used commercial bases." While this observation is of considerable interest, their base is not satisfactory from the standpoint of the present investigation since one of its expressed purposes is to develop a lanolin-free base. Accordingly, the substances listed in Table V were studied in relation to their contributions to the stability of the emulsions prepared from the cholesterol-cholesteryl stearate base. No emulsifying efficiency studies were made on these bases. Each emulsion was prepared by incorporating 50 Gm. of distilled water in 10 Gm. of the appropriate base. The water thus represents 500% of the base or 83 1/3% of the finished emulsion, probably more than is usually needed or desired in preparations of this type.

Soybean Lecithin.—The value of soybean lecithin in stabilizing cholesterol and oxycholesterol emulsions has been emphasized by Redgrove (6). It has been shown above that the lecithin enhanced the emulsifying efficiency of the cholesterol-cholesteryl stearate mixture but that the emulsions containing very large amounts of water were not stable on shelf testing. However, when the amount of water was reduced to 83 1/3% of the finished emulsion, the stability of the products was much more satisfactory. The addition of other substances such as cetyl alcohol, further increased the stability. In addition, the lecithin seemed to improve the texture and skin test qualities of each emulsion and, as mentioned above, increased the speed of emulsification. The most serious difficulty noted in the use of soybean lecithin was the tendency of all emulsions containing it to darken when exposed to direct sunlight.

Cetyl Alcohol.—Atkins (7), in his excellent review, has summarized the advantageous features of cetyl alcohol and has called attention to its wide use in stabilizing emulsions prepared from absorption bases. Preliminary experiments indicated that the addition of cetyl alcohol to the bases under discussion definitely improved the stability of the emulsions prepared from them and imparted a finer grain to the products. For the most part, those emulsions containing cetyl alcohol possessed relatively good shelf test and skin test qualities. The velvety texture imparted to the skin by cetyl alcohol is, of course, of considerable cosmetic importance.

Cetyl 12-Hydroxy Stearate.—Emulsions 24 and 25, containing this ester, exhibited poor shelf test stability and the skin test qualities were inferior to those of emulsions 22 and 23, which contained cetyl alcohol.

Sorbitol.—In a separate experiment it was shown that sorbitol, unlike glycerin, did not appreciably lower the emulsifying efficiency of the cholesterol-cholesteryl stearate base nor did it seriously impair the stability. However, when used in combination with soybean lecithin and cetyl alcohol, sorbitol produced completely unsatisfactory emulsions which softened upon standing for four or five days and which liquefied when applied to the skin.

Glyceryl Monostearate and Glyceryl Monoricinoleate.—The existence of dual emulsion systems is well established (8) and it was thought that the production of such systems might lead to greater stability. However, when these normally oil-in-water promoting emulsifying agents were added to the cholesterol-cholesteryl stearate base in the amounts indicated, no emulsions were produced. Further investigation of this phase of the problem is now in progress and will be the subject of a future communication.

Other Substances.—With the possible exceptions of diglycol stearate and heavy mineral oil, the other substances studied proved unsatisfactory in stabilizing the emulsions.

SUMMARY

1. The addition of cholesteryl esters of acetic, butyric, caproic and lauric acids to a solution of cholesterol and cholesteryl stearate in white petrolatum resulted in a decrease in emulsifying efficiency in all instances. Cholesteryl palmitate did not decrease the efficiency.

2. Soybean lecithin enhanced the emulsifying efficiency of a solution of cholesterol and cholesteryl stearate in white petrolatum, sorbitol had virtually no effect, while glycerin impaired the efficiency.

3. Soybean lecithin and cetyl alcohol improved the shelf test and skin test qualities of emulsions of distilled water in white petrolatum promoted by a mixture of cholesterol and cholesteryl stearate. The addition of sorbitol to such emulsions resulted in softening upon standing and liquefaction upon application to the skin. Cocoa butter, diglycol stearate, castor oil, hydrogenated castor oil, heavy mineral oil, carbitol and triacetin were of little or no value in enhancing the stability of such emulsions.

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The Identification of a Diol Isolated from Uva Ursi*

By Jack Edward Orr,† Lloyd M. Parks,‡ Melvin F. W. Dunker and Arthur H. Uhl

The isolation of a high molecular weight alcohol from *Arctostaphylos Uva Ursi* (Linné) Sprengel has been described by Parks (1). This compound was not completely characterized. However, an acetate was prepared, its saponification equivalent determined, and an approximate carbon and hydrogen analysis of the free alcohol made. From its properties, Parks assumed at that time (1937) that this alcohol might be a new representative of a group of polycyclic alcohols commonly referred to as triterpene alcohols. This assumption was strengthened by the fact that ursolic acid, a triterpene hydroxy-acid, exists in Uva Ursi, and it is not inconceivable that the unknown alcohol might be a decarboxylation or a reduction product of ursolic acid. The possibility that this alcohol might be a mono-hydroxy-triterpenoid resulting from the decarboxylation of ursolic acid or, possibly, of its isomer, oleanolic acid, was shown to be unlikely since the constants reported in the literature for the decarboxylation products of these acids did not agree with those of the Uva Ursi alcohol.

The melting point of the alcohol was, however, close to that of a diol later prepared by Ruzicka (2) by reduction of ursolic aldehyde, and to erythrodiol, a diol comparing to oleanolic acid in the same way. It was therefore deemed more likely that the Uva Ursi alcohol was a diol. In the same year Huzii and Ōsumi (3) also reported a diol prepared from ursolic acid by reduction of the phenyl acetyl ester with sodium and amyl alcohol. They further showed their diol to be identical with a diol which they called "uvaol," isolated from Uva Ursi and from *Leucothoe Keiskei* Miq. It is probable that uvaol and the Uva Ursi alcohol herein described are the same. If so, this work confirms theirs.

The work reported here was undertaken for the purposes of purifying the Uva Ursi alcohol and establishing its identity. The source of the unknown alcohol was a crude petroleum ether extract of Uva Ursi leaves obtained by Feiertag (4). This material, which was shown to consist of a mixture of the alcohol and ursolic acid, was purified by crystallization, by extraction with sodium hydroxide to remove ursolic acid, by crystallization of the acetate and saponification back to the free alcohol. Analyses of the free alcohol and its acetate, as well as determination of the saponification equivalent of the acetate, showed that the Uva Ursi alcohol was a diol with the probable molecular formula, $C_{30}H_{50}O_2$. The diol obtained by reduction of methyl ursolate with sodium and ethanol, and its diacetate were shown to be identical with the Uva Ursi diol and its diacetate. The results also indicated that this diol was probably the same as that prepared by Ruzicka from ursolic aldehyde. The constants of the diol and its diacetate were somewhat lower than those of uvaol (see Table I).

Since oleanolic acid was at hand, it was reduced to the diol through its methyl ester in order to obtain a sample of erythrodiol for comparison with the diol isolated. Erythrodiol is a triterpene diol, $C_{30}H_{50}O_2$, occurring naturally in *Erythroxylon novogranatense* Morren (5). It has also been prepared from oleanolic acid by Ruzicka (6) as a by-product of a Rosenmund reduction of the acid chloride followed by a Wolff–Kishner reduction of the aldehyde obtained. The method described in this paper has not previously been reported for this conversion. The product obtained was apparently identical with erythrodiol, but not with the Uva Ursi diol.

EXPERIMENTAL[1]

Purification of the Uva Ursi Diol.—The starting material, a light, fluffy, greenish powder melting at 203–221° C. (uncorr.), was a crude Skellysolve "B" extract of powdered Uva Ursi leaves. It was purified by several crystallizations from ethanol and methanol and by removal of the contaminating ursolic acid as its sodium salt which is insoluble in both ether and water. Crystallization of the isolated alcohol from acetone gave fine, white needles, m. p. 222–223° C.

$[\alpha]_D^{24}$ + 68.57° (56 mg. in 2 ml. $CHCl_3$, α_D + 1.92°, *l* 1 dm.).

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[1] Unless otherwise indicated, all melting points are corrected by comparison against a Bureau of Standards calibrated set of Anschutz thermometers.

Anal. Calcd. for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38. Found: C, 80.28, 80.41; H, 10.66, 10.56.

The low results in the analyses were probably due to small amounts of ursolic acid contaminating the sample.

Diacetate of the Uva Ursi Diol.—The diol was acetylated by the method of Drake and Shrader (7) using 176.5 mg. of the diol in 16 ml. of pyridine and 2 ml. of acetic anhydride. Crystallization from ethanol and from acetone gave 93 mg. (57%) of white needles, m. p. 150.4–151.4° C. The analytical results agreed well with the theoretical for a diacetate.

$[\alpha]_D^{25}$ + 53.36° (52.1 mg. in 2 ml. benzene, α_D + 1.39°, *l* 1 dm.).

Anal. Calcd. for $C_{34}H_{54}O_4$: C, 77.52; H, 10.33. Found: C, 77.35; H, 10.49.

and 13 Gm. of sodium, a ten mole excess, was added. About one mole equivalent, 4 ml., of absolute ethanol was dropped in from the funnel during fifty minutes of refluxing on a steam bath. The mixture was refluxed for another forty minutes, 95% ethanol added until the solution became clear, and the solution refluxed fifty minutes longer. It was filtered and the filtrate washed with water, 10% hydrochloric acid, and again with water. The ether solution was dried and solvent removed. Crystallization of the crude product from ethanol gave 520 mg. of white, needle crystals, m. p. 221.5–223° C., and 476 mg., m. p. 223–224.5° C. Yield: 62.5%. Crystallization of the combined fractions from acetone and from methanol gave a constant m. p. at 223–224° C. A mixed melting point with the Uva Ursi diol showed no depression.

TABLE I.—COMPARISON OF PROPERTIES OF THE DIOLS

	Uva Ursi Diol	Synthetic Diol	Ruzicka's Diol (2)	Uvaol (3, 8)
Free diol, m. p.	225.5–226.5° (No depression when mixed)	223–224°	223–226°	232–233°
$[\alpha]_D$	+70.88° ($CHCl_3$)	+68.80° ($CHCl_3$)	+71.33° ($CHCl_3$)	+74.4° ($CHCl_3$)
Diacetate, m. p.	150.4–151.4° (No depression when mixed)	153.6–154.6°	150–151°	157–159°
$[\alpha]_D$	+53.36° (benzene)	+54.08° (benzene)	+53° (benzene)	Not reported

Saponification of the Diacetate.—The above diacetate was saponified by refluxing with alcoholic potassium hydroxide for twenty-four hours. The product was worked up in the usual way and, after several crystallizations from ethanol and from methanol, melted at 225.5–226.5° C.

$[\alpha]_D^{25}$ + 70.88° (44.3 mg. in 2 ml. $CHCl_3$, α_D + 1.57°, *l* 1 dm.).

The diol thus appeared to have been purified somewhat through its diacetate. However, not enough was available for analysis.

Methyl Ursolate.—To 3 Gm. (0.0065 mole) of ursolic acid dissolved in 90 ml. of absolute methanol was added 8.7 ml. of a methyl alcoholic solution of potassium hydroxide containing 0.05 Gm./ml. To this was added 1.04 Gm. (0.0083 mole) of dimethyl sulfate and the mixture was refluxed for four and one-half hours on a steam bath. The mixture was poured into water and a heavy, white precipitate formed. This was filtered off, dried, dissolved in ether and the ether solution washed three times with 100-ml. portions of 3% aqueous sodium hydroxide then once with water. During the alkali washings, a considerable amount of unreacted acid was precipitated as its sodium salt. This was removed and the ether evaporated. A yield of 35% of product was obtained, m. p. 169–169.5° C., after crystallization from ethanol.

Other runs yielded 72.5% and 50% of methyl ursolate when the amount of alkali present was reduced. It was also found that yields could be materially increased by refluxing for a longer period combined with addition of more dimethyl sulfate and alkali at intervals.

Reduction of Methyl Ursolate.—A solution of 1.7 Gm. of methyl ursolate in 100 ml. of Skellysolve "B" was placed in a three-necked, round-bottomed flask fitted with a reflux condenser, a mechanical stirrer with a mercury seal, and a dropping funnel,

$[\alpha]_D^{25}$ + 68.80° (52 mg. in 2 ml. $CHCl_3$, α_D + 1.72°, *l* 1 dm.).

Anal.—Calcd. for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38 Found: C, 80.42, 80.41; H, 11.06, 10.78.

The low values obtained are probably due to small amounts of impurities. Although the values are somewhat low, it will be seen that they check quite closely with those observed for the Uva Ursi diol.

Diacetate.—A sample of 170 mg. of the diol melting at 223–224° C. was acetylated by the method of Drake and Shrader (7) and the product worked up in the usual manner. Yield: 130 mg. of snow-white needles, m. p. 153.6–154.6° C. A mixed melting point with the Uva Ursi diol diacetate showed no depression.

$[\alpha]_D^{25}$ + 54.08° (51.4 mg. in 2 ml. benzene, α_D + 1.39°, *l* 1 dm.).

These results show conclusively that the Uva Ursi diol is identical with that synthesized from ursolic acid and probably with Ruzicka's diol. It is also probable that it is the same as that isolated by Huzii and Ōsumi (8).

Methyl Oleanolate.—To 20 Gm. (0.044 mole) of oleanolic acid dissolved in 300 ml. of absolute methanol were added 2.9 Gm. of potassium hydroxide and 6.5 Gm. (0.052 mole) of dimethyl sulfate, and the mixture refluxed on a steam bath for four and one-half hours. The product was worked up in the same way as described for methyl urosolate except that the unreacted acid which was precipitated by the alkali washings was remethylated and combined with the main product. Crystallization from ethanol gave three crystal crops melting at 201–202° C., 200° C., and 198–199° C., a total of 16.4 Gm. (82%).

Further quantities of the ester were prepared by the same method with yields of 60–65%. The unreacted oleanolic acid was not re-methylated in these runs.

Hydrogenation of Methyl Oleanolate.—A solution of 16.4 Gm. (0.035 mole) of methyl oleanolate in 200 ml. of dioxane was hydrogenated[2] over a catalyst of 3 Gm. of copper chromite for six hours under 300 atmospheres pressure at 260° C. The compound consumed approximately 0.10–0.12 mole of hydrogen. The amount calculated as necessary to reduce the ester linkage to an hydroxyl group without affecting the double bond is 0.07 mole. Since oleanolic acid itself resists attempts to hydrogenate its double bond, it was not expected that the double bond would be reduced in its methyl ester. A total yield of 14.68 Gm. was obtained, 89.1% calculated as the diol which would be expected to be formed by reduction of the ester grouping without reduction of the double bond.

Crystallization of the product yielded two fractions of material melting at 215–220° C. and at 203° C. Further crystallizations and a series of chromatographic adsorptions on aluminum oxide of the various crystal crops yielded only a small amount of crystals with a sharp melting point at 231–232° C.

Since only a very small amount of high-melting, seemingly pure product was obtained in this run, the hydrogenation was repeated. A sample of 9.95 Gm. (0.021 mole) of methyl oleanolate was hydrogenated as before. It apparently consumed 0.06–0.07 mole of hydrogen as compared to a theoretical of 0.042 mole. A yield of 9.4 Gm. (94.5%) of crude product was obtained, m. p. 204.8–210.7° C.

Acetylation of the Hydrogenation Products.—Purification of the hydrogenation product itself in the first run had proved difficult, wasteful, and time-consuming; therefore, after a preliminary crystallization it was decided to attempt purification through the acetates. Fractions of the crystal crops from run 2 were acetylated separately and several fractions, all melting near each other, from both runs were combined and acetylated to produce acetates having the following properties in yields of 85–94%:

1. M. p. 182.5–183° C., $[\alpha]^{25.5}_{D}$ + 56.61° (54.4 mg. in 2 ml. $CHCl_3$, α_D + 1.54°, l 1 dm.).
2. M. p. 177–178° C.
3. M. p. 175.5–177° C.
4. M. p. 181–182° C., $[\alpha]^{25.5}_{D}$ + 60.43° (41.7 mg. in 2 ml. $CHCl_3$, α_D + 1.26°, l 1 dm.). *Anal.* Calcd. for $C_{34}H_{54}O_4$: C, 77.52; H, 10.33. Found: C, 76.70; H, 10.16.

Although low, the analytical results agree fairly well with the theoretical for a diacetate. This indicates that the original hydrogenation product was probably a diol. This fact was also indicated by a determination of the saponification equivalent of acetate 3. The method of Shriner and Fuson (9) was used and the results indicated a saponification equivalent of 266. Assuming the presence of one hydroxyl group in the molecule, the molecular weight of the acetate would be the same as the saponification equivalent. If it is assumed that two hydroxyl groups are present, the molecular weight of the diacetate would be twice the value obtained. The diacetate of the diol which would be expected to form by reduction of methyl oleanolate would have a molecular weight of 526. The result obtained, when multiplied by two, is in fair agreement with this value.

Saponification of the Acetates.—Acetate No. 1, above, was saponified by refluxing with methyl alcoholic potassium hydroxide. After working up the product in the usual way, crystallization from methanol gave crystals melting at 230.5–231.5° C.

$[\alpha]^{24.5}_{D}$ + 66.66° (50.1 mg. in 2 ml. dioxane, α_D + 1.67°, l 1 dm.).

Acetate No. 4 was saponified as above and the product, after crystallization from ethanol and methanol, melted at 229.5–230.5° C.

$[\alpha]^{25.5}_{D}$ + 69.29° (38.1 mg. in 2 ml. $CHCl_3$, α_D + 1.32°, l 1 dm.).

TABLE II.—COMPARISON OF THE HYDROGENATION PRODUCT WITH ERYTHRODIOL

	Hydrogenation Product	Erythrodiol	
		Zimmermann	Ruzicka
Free diol, m. p.	231–232°	231°	232–233°
$[\alpha]_D$	+69.29° ($CHCl_3$)	+75.38° ($CHCl_3$)	+74.6° ($CHCl_3$)
Diacetate, m. p.	182.5–183°	188°	184–185°
$[\alpha]_D$	+60.43° ($CHCl_3$)	+59.41° ($CHCl_3$)	+60° ($CHCl_3$)
Diformate, m. p.	194.5–195.5°	195°	

Preparation of a Diformate.—A diformate of the diol was prepared by refluxing 0.5 Gm. with 85% formic acid for ten minutes. The product was worked up in the usual way and then crystallized from methanol. Fine, white platelets were obtained, m. p. 190–193° C. Recrystallization from ethanol raised the m. p. to 195–198° C. Two recrystallizations from acetone continued to raise the melting point and widen the melting range, indicating that decomposition was taking place. Concentration of the acetone mother liquors gave crystals melting sharply at 194.5–195.5° C. Further crystallizations from acetone continued to raise the melting point.

Repetition of the formylation gave similar results, *i. e.*, crystals melting sharply around 195° C. and then melting higher after each crystallization. The 195° C. melting point corresponds to that for the diformate of erythrodiol prepared by Zimmerman (10).

Examination of the results listed in the above table indicates that the diol prepared from oleanolic acid very likely is identical with erythrodiol. Comparison of the melting point of the diacetate with that of the diacetate of the Uva Ursi diol indicates that the two are not identical since there is a 30–35° variation.

A search of the literature revealed that erythrodiol has not previously been prepared from oleanolic acid by catalytic hydrogenation.

SUMMARY AND CONCLUSIONS

1. A high molecular weight alcohol isolated from *Arctostaphylos Uva Ursi* (Linné) Sprengel has been shown to be a triterpene diol, $C_{30}H_{50}O_2$.

[2] The authors wish to express their thanks to Prof. Homer Adkins for carrying out the catalytic hydrogenations.

2. Ursolic acid has been converted to this diol, establishing the structural relationship between the two.

3. Oleanolic acid has been converted into the triterpene diol, erythrodiol, by catalytic hydrogenation of its methyl ester.

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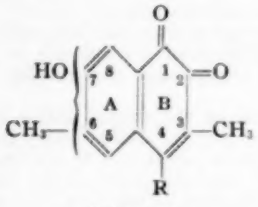
The Synthesis of 2-Hydroxyisophthalic Acid and Some of Its Derivatives*,†

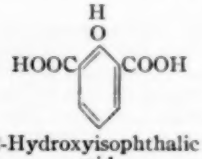
By William S. Benica and Ole Gisvold‡

Oxidative studies carried out upon the pigment celastrol, a naturally occurring hydroxy β-naphthoquinone, yielded a small quantity of crystalline material that melted at about 244° C., and gave a positive phthalein test. This acid fraction, however, failed to give a color reaction with ferric chloride T. S. Because celastrol is a β-naphthoquinone and has a free hydroxyl group a number of monohydroxy phthalic acids are possible. This number is increased if ring *A* contains a methyl group that is oxidized to a carboxyl group followed by the loss of a carboxyl group having its origin from the ring *B*.

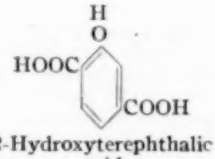


Celastrol

If the hydroxyl group is at 8-position and the methyl group at position 7, oxidation followed by decarboxylation should yield on of the following hydroxy phthalic acids:



2-Hydroxyisophthalic acid



2-Hydroxyterephthalic acid

The melting point of 2-hydroxyisophthalic acid has been reported as 230° C. by Graebe and Kraft (1), 239° C. by Reimer and Tiemann (2), 239°–240° and 242° C. by Hasse (3), 240°–243° C. by Jacobsen (4) and 243° C. by Hähle (5). The only authors who specifically state that this is the melting point of the hydrated form of the acid are Reimer and Tiemann. Schall (6) reported the melting point of air-dried 2-hydroxyisophthalic acid as 239° C. in the experimental part of his publication and as 242° C. in the summary. Hähle (5) states that the acid can exist in an anhydrous form or in a form containing 1 mole of water of crystallization, but does not state to which form his reported melting point of 243° C. refers. The only reported melting point of the anhydrous acid is 244° C. (2).

Schall (6) reported that a cherry-red color resulted from the addition of a solution of ferric chloride to an aqueous solution of the acid. Jacobsen (4) reported that a red-violet color resulted from the same reaction, and Hähle (5) reported a carmine color.

* Received Aug. 28, 1944, from the College of Pharmacy, University of Minnesota, Minneapolis, Minn.

† Abstracted from a thesis submitted by W. S. Benica to the graduate faculty of the University of Minnesota in partial fulfillment for the requirements for the degree of Master of Science.

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Reimer and Tiemann (2) and Schall (6) reported that neutral solutions of 2-hydroxyisophthalic acid show a blue fluorescence.

The purest samples of 2-hydroxyisophthalic acid prepared in this investigation when air dried were fine, colorless, hexagonal needles that melted at 244.5°–245° C. When dried they melted at 250°–250.5° C. Aqueous and alcoholic solutions of these crystals showed a blue-violet fluorescence. A saturated aqueous solution of 2-hydroxyisophthalic acid gave a light reddish orange color with ferric chloride test solution. A mixed melting point of this acid with the oxidation fragment from celastrol gave a marked depression of the melting point. The two compounds behave quite differently in their chemical and physical properties with the exception of their melting points.

EXPERIMENTAL

The method of Hasse (3) which involves a direct synthesis of 2-hydroxyisophthalic acid from salicylic acid by a modified Reimer–Tiemann reaction using carbon tetrachloride, copper powder and aqueous sodium hydroxide gave negligible yields.

The method of Reimer and Tiemann (2) which involves the preparation of 3-aldehydo salicylic acid as an intermediate gave very small yields of the 3-aldehydo compound whereas the main product was 5-aldehydo salicylic acid. Attempts at the conversion of the 3-aldehydo salicylic acid to 2-hydroxyisophthalic acid by permanganate oxidation or potassium hydroxide fusion failed.

Graebe and Kraft (1) claimed an 80 per cent yield of 2-hydroxyisophthalic acid by the oxidation of orthocresotinic acid by means of potassium hydroxide and lead dioxide. This procedure was used with the following results. Twenty grams of orthocresotinic acid (0.131 mole), 120 Gm. of potassium hydroxide (2.14 moles) and 100 Gm. of lead dioxide (0.4 mole) were mixed in a 1000-cc. Pyrex beaker with 24 cc. of water and the mixture heated to 225°–240° C. The dark brown pasty mass became red and more fluid, and after twelve minutes the liquid boiled up and then subsided. As it subsided it turned dark greenish brown and a pellicle formed on the surface. The heating was continued for forty minutes. The mass was allowed to cool and was then boiled with 400 cc. of water to remove the water-soluble material. This solution was then filtered and acidified with concentrated hydrochloric acid until the precipitated material, which was yellow at first, turned a light tan color. This mixture was shaken with 40 cc. of chloroform and the whole filtered through a sintered glass funnel with the aid of suction. The chloroform layer of the filtrate was then separated and evaporated on the steam bath. The residue was a viscous, dark liquid with a strong phenolic odor suggestive of cresols. It was not investigated further. The solid material that was collected in the sintered glass funnel was heated to boiling with 1 liter of water and 20 Gm. of "Norite." The hot mixture was filtered through a steam-heated funnel and when allowed to stand overnight deposited crystalline material. This material was collected on a filter and dissolved in 600 cc. of boiling water containing 15 cc. of concentrated sulfuric acid. The mixture was allowed to cool to 70° C. and then filtered. The material that separated between the boiling point and 70° C. consisted almost entirely of unchanged orthocresotinic acid. The clear filtrate upon cooling in the refrigerator deposited crystals which were collected and dissolved in 180 cc. of ether. The ether was concentrated to a volume of 20 cc. mixed with 300 cc. of water, and heated on a steam bath to remove the ether and dissolve the crystalline material. The hot solution was then allowed to cool to room temperature. Three and nine-tenths grams (14.8%) of crystals were obtained, m. p. 238°–239° C.

The above procedure as carried out in a Pyrex beaker did not give very good yields because of the rapid attack of the glass by the alkali. This difficulty was overcome by using a 1000-cc. copper beaker. The best yield using this type of a container was 11.0 Gm. of 2-hydroxyisophthalic acid, m. p. 242° C.

The 2-hydroxyisophthalic acid, although having a melting point comparable to that reported in the literature, was purified as follows: The free acid was precipitated from a hot aqueous solution by the addition of a solution of lead acetate. The precipitate was washed and then decomposed with a slight excess of boiling aqueous sulfuric acid. The mixture was cooled to 70° C. and filtered. The filtrate upon cooling deposited needle-like crystals which, after several recrystallizations from water and air dried, melted at 244.5°–245° C. Further recrystallizations failed to change the melting point. A sample of this product when dried in an Abderhalden drying pistol under a pressure of 3.4 mm. of Hg for six hours at 110° C. produced an anhydrous crystal, m. p. 250°–250.5° C.

Pure crystals of 2-hydroxyisophthalic acid containing 1 molecule of water of crystallization are fine, colorless hexagonal needles often 2 cm. long. Aqueous and alcoholic solutions of these crystals show a blue-violet fluorescence which disappears on the addition of excess acid or alkali. The dry crystals show a beautiful, pale blue fluorescence in the presence of ultraviolet light. A saturated aqueous solution gave a light, reddish orange color with ferric chloride T. S.

Dimethyl Ester of 2-Hydroxyisophthalic Acid.—Attempts to methylate the free carboxyl groups with diazomethane did not produce very desirable results. A small amount of the dimethyl ester, m. p. 70° C., was obtained. Therefore, 1 Gm. of the acid was dissolved in 30 cc. of methyl alcohol containing 2 per cent hydrochloric acid (1.2 cc. of concentrated hydrochloric acid), and the solution refluxed for four hours. The solution was then cooled and neutralized with solid sodium bicarbonate. The insoluble material was filtered off and the filtrate diluted with 200 cc. of water. The milky solution was extracted with three 25-cc. portions of ether. The ether was removed on the steam bath and the residue crystallized from dilute methyl alcohol. They melted at 70° C., yield 0.18 Gm. or 17.1 per cent. The above procedure was repeated using 2.5 cc. of concentrated sulfuric acid instead of the hydrochloric acid, m. p. 71° C., yield 2.6 Gm., 82.5 per cent.

The dimethyl ester was also prepared by treating the silver salt of the acid with methyl iodide, m. p. 71° C., yield 0.18 Gm., 34 per cent.

The diethyl ester was prepared in the same manner as the dimethyl ester, sulfuric acid being used as the condensing agent. This derivative was crystallized from dilute ethyl alcohol, m. p. 112° C.; yield 0.73 Gm., 61 per cent.

Diparabromphenacyl Ester.—The method used for the preparation of this type of derivative was that described by Reid and Judefind (7). One gram (0.005 mole) of 2-hydroxyisophthalic acid contained in a 100-cc. round-bottom flask was almost neutralized by 9.9 cc. of normal potassium hydroxide solution. To this were added 10 cc. of 95 per

cent ethyl alcohol and 2.70 Gm. of parabromophenacyl bromide (0.010 mole). This mixture was refluxed for half an hour, 30 cc. of 50 per cent ethyl alcohol was added and heating continued for another hour and a half. The solution was filtered while hot and then allowed to cool. The crystalline material which separated was recrystallized from dilute acetone. White plates were obtained, m. p. 162° C.

Anal.—Calcd. for $C_{24}H_{16}O_7Br_2$: Br, 27.8. Found: Br, 29.1.

Monoparabromphenacyl Ester.—In the preparation of the above di-ester, a precipitate was obtained after the mixture had refluxed for about ten minutes. The reaction mixture was allowed to cool and the solid material that had separated was collected and recrystallized from dilute ethyl alcohol, m. p. 196° C.

Anal.—Calcd. for $C_{16}H_{19}O_6BrK$: Br, 19.2. Found: 20.1.

This derivative when acidulated produced the free acid half-ester which melted at 109° C.

Acetyl Derivative—One gram of 2-hydroxyisophthalic acid, 2 cc. of acetic anhydride and 1 drop of concentrated sulfuric acid were heated on the steam bath for twenty minutes. The mixture was cooled and the derivative which separated was collected and recrystallized from acetone, m. p. 163° C.

Nitro-Derivative—One gram of 2-hydroxyisophthalic acid was mixed with 10 cc. of concentrated nitric acid and heated on the steam bath. Solution took place quite readily and a vigorous evolution of the oxides of nitrogen occurred. When this evolution subsided, the mixture was heated an additional ten minutes on the steam bath and then placed in the refrigerator. The crystals which separated were collected and recrystallized from hot water with considerable loss of material. Fine, white needles were obtained which melted sharply at 187° C. Aqueous solutions of this derivative were yellow and nonfluorescent. This nitro compound no doubt contains the nitro group in position 5.

Amino-Derivative—Two grams of the above nitro compound were dissolved in 400 cc. of 50 per cent aqueous alcohol and 0.5 Gm. of Raney nickel added. Reduction was carried out at room temperature for an hour at a pressure of 38 lb. of hydrogen. The solution was filtered to remove the catalyst and the filtrate evaporated on the steam bath to dryness. Twenty-five cubic centimeters of dilute hydrochloric acid were added to the dark residue and the mixture heated to boiling. A small amount of "Norite" was added to the boiling liquid and the mixture filtered. The solution was placed in the refrigerator and colorless crystals separated. After recrystallization from hot water 0.086 Gm. (4.95%) of crystals was obtained that melted at 273° C. with decomposition.

SUMMARY

1. The melting points of hydrous and anhydrous 2-hydroxyisophthalic acid have been found to be higher than those previously reported in the literature.

2. Pure 2-hydroxyisophthalic acid in a saturated aqueous solution gave a reddish orange color with ferric chloride T. S. The color reaction with ferric chloride is reported in the literature to be cherry-red, red-violet and carmine.

3. The following previously unreported derivatives of 2-hydroxyisophthalic acid have been prepared: diethyl ester, monoparabromphenacyl ester of the free acid and its potassium salt, diparabromphenacyl ester, acetyl compound, 5-nitrocompound and the 5-amino compound.

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(5) Hähle, H., *J. prakt. Chem.*, **44,** 6(1891).
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WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

Allyl cyanamide
Afridol
Adenosine-5-pyrophosphate
5-Butyl-5-isoamyl barbituric acid
Cytosine
p-Chlorophenyl acetic acid
Cyclogallipharic acid
Desoxy ribose
2,3-Dihydroxy naphthalene-6-sulfonic acid
Gheddaic acid
Glutarenghol
Hydrocupreine sulfate
Norvaline
Pelandjauic acid
Phylloporphyrin
Promin
Renghol
Sodium amylopectin glycollate
Stilbamidine
2,6,7-Trihydroxy-9-methyl-3-isoxanthone

A Study of Some of the N. F. VII Requirements for Areca*

By Charles L. Williams† and L. David Hiner‡

The National Formulary in its description of unground areca seeds states that they are "round conical," up to 3 cm. in length and 2 cm. in diameter, up to 5 Gm. in weight (1) and continues further with the description of their physical appearance. The statement regarding the weight limit attracted the attention of Dr. E. H. Wirth and others of the National Formulary Subcommittee (2), to the extent that each took samples at random from their stocks and weighed the individual seeds, obtaining varied results. It was agreed that more extensive work should be done in this respect to determine whether the weight requirement was within reason, whereupon the problem was assigned to L. D. Hiner, who in turn selected Myrdas P. Brewer to do the experimental work.

EXPERIMENTAL

The seeds for Mrs. Brewer's experimental work were obtained by taking small samples at random from the stock packages at hand which, when combined, totaled 62 seeds. Of this number, 24 were 5 Gm. or under; and the remainder were in excess of the present N. F. 5-Gm. maximum-weight limit.

These seeds were divided into three distinct sizes: those weighing about 3 Gm., those weighing about 9 Gm., and those weighing about 5 Gm. They were then ground, passed through a number 40 sieve and assayed for the ether-soluble alkaloids by the process outlined in N. F. VII. This division of the sample did not leave very large quantities with which to work; and, as a result, no definite conclusions were drawn.

Since Mrs. Brewer's work was done on such a small quantity of seeds, it was evident that more conclusive results could be obtained if much larger amounts were used. Accordingly 10 pounds of commercial, unground areca were secured.[1]

All seeds which had been attacked by insects or any that were broken, were discarded. The remainder were divided into lots of those weighing 5 Gm. and less, +5 to 6 Gm., +6 to 7 Gm., +7 to 8 Gm., +8 to 9 Gm., +9 to 10 Gm., +10 to 11 Gm., + 11 to 12 Gm., +12 to 13 Gm., +13 to 14 Gm., and +14, respectively. The results obtained are shown in Table I.

The total weight of 547 seeds was 4346.67 Gm., and the average weight was 7.767 Gm. for the individual seeds.

The weights (Table I) show that from a total of 547 seeds, 541 of them, or 98.9%, would not meet the present N. F. weight requirement. On the other hand, of the 4346.67 Gm., by weight, 4324.22 Gm., or 99.48%, would be rejected because of the N. F. weight requirement.

These results seemed to indicate that the N. F. VII weight requirement for areca should be modified or even eliminated; but, in order to confirm this observation, it was necessary to assay samples of each lot. Since there was not a sufficient amount of some lots to furnish enough samples for assay purposes, Groups II through VIII, inclusive, only were used. Approximately 150 Gm. of each were ground separately and passed through a number 40 sieve.

Eight samples of each lot were weighed accurately and assayed by the procedure described in the N. F. VII with the results shown in Table II.

TABLE I.—WEIGHT OF SEEDS

Group	No. of Seeds	Seed Size Range by Weight	Total Weight, Gm.	Average Weight, Gm.
I	6	5 Gm. and less	22.45	3.74
II	28	+5 Gm. to 6 Gm.	155.93	5.56
III	113	+6 Gm. to 7 Gm.	746.92	6.61
IV	193	+7 Gm. to 8 Gm.	1450.11	7.51
V	101	+8 Gm. to 9 Gm.	896.95	8.86
VI	66	+9 Gm. to 10 Gm.	622.19	9.43
VII	19	+10 Gm. to 11 Gm.	196.95	10.36
VIII	13	+11 Gm. to 12 Gm.	148.68	11.44
IX	2	+12 Gm. to 13 Gm.	25.62	12.81
X	5	+13 Gm. to 14 Gm.	66.30	13.26
XI	1	+14 Gm.	14.57	14.57

TABLE II

Group	Seed Size Range by Weight	Strength of Ether-Soluble Alkaloids Calculated as Arecoline, Av. %
II	+5 to 6 Gm.	0.449
III	+6 to 7 Gm.	0.453
IV	+7 to 8 Gm.	0.452
V	+8 to 9 Gm.	0.457
VI	+9 to 10 Gm.	0.450
VII	+10 to 11 Gm.	0.453
VIII	+11 to 12 Gm.	0.455

The results of these assays show that there is very little difference, if any, in the alkaloidal content of the various weight groups. They show that all samples are above the N. F. VII alkaloidal requirement. The combined results of this work show conclusively that the N. F. VII 5-Gm. maximum-weight limit is definitely unwarranted for these ordinary commercial areca samples.

Since the N. F. VII in its description also stated definite measurements, namely "up to 3 cm. in length and 2 cm. in diameter" (1), it was decided to check up on these requirements also. As a result, the entire 547 seeds were measured with a micrometer caliper to the second decimal. Only 10, or 1.83% of the 547 seeds measured, came within the present N. F. VII measurement requirement.

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[1] Acknowledgment is made to Dr. G. M. Hocking of S. B. Penick and Company for samples of areca.

Two seeds exceeded the length requirement, while all, with the exception of the ten which met the N. F. VII measurement requirements, exceeded the diameter requirement. It is significant that from the number of seeds which met the N. F. VII requirements for size, only three came within the official weight requirement; therefore, on the basis of both size and weight, there were only three N. F. seeds in the entire lot. The three N. F. seeds weighed a total of 9.44 Gm. Table III shows the average dimensions of each group.

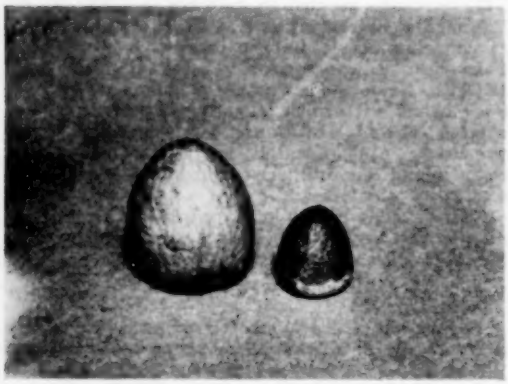

Fig. 1.—Approximately actual size.
The small seed on the right weighed 3.45 Gm. and meets the N. F. VII requirements, while the large seed on the left weighed 14.57 Gm. and as a result would be rejected according to the present N. F. VII standards for size and weight. Both samples met the N. F. VII assay requirements.

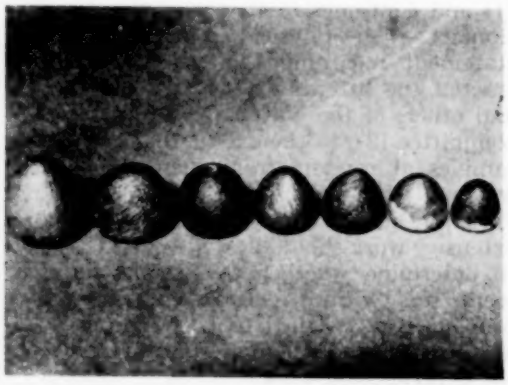

Fig. 2.—Approximately 1/2 actual size.
The small seed on the right represents the N. F. VII accepted sample while all of those to the left ranging from +5 Gm. to +14 Gm. would have been rejected by the present N. F. VII requirements.

Table III is conclusive evidence that the N. F. measurement requirement is inaccurate for present commercial samples of areca. It shows the average diameter to be greater than the average length, and it also shows that the diameter requirement caused all but about 0.6% of the seeds to be rejected.

As a further check, a representative sample of Groups II to VIII, inclusive, was made by taking 4 Gm. from each sample and mixing them thoroughly; then four ash determinations were made of this mixture by the U. S. P. XII method for total ash in vegetable drugs (3). The results showed this sample to be 1.53% total ash.

It was noted that the N. F. VII requires that a moderately fine powder be used in the assay process, yet neither the U. S. P. nor the N. F. describes such a powder; therefore, a moderately coarse powder (No. 40) was used in this work with satisfactory results.

In the assay process for areca one deviation from the N. F. VII method is recommended. Every step should be followed precisely until the N. F. directs that the remaining ether be extracted in a separator. Instead of transferring the ether solution to a separator and adding the fiftieth-normal sulfuric acid, it is suggested that the acid be added directly to the solution in the distilling flask which is then placed in the shaker and shaken for ten minutes. Then pour the menstruum into the separator; wash each flask with three successive 5-cc. portions of distilled water, which is then passed through the separator. This is much easier and requires less time, because as many as 16 samples can be shaken simultaneously. The final results obtained by this method checked well within the tolerable limits, thereby indicating that the N. F. should recommend or permit such a deviation from its assay process where the apparatus is available.

TABLE III

Group	No. of Seeds	Seed Size Range by Weight	Average Diameter, Cm.	Average Length, Cm.
I	6	5 Gm. and less	2.05	1.92
II	28	+5 Gm. to 6 Gm.	2.21	2.03
III	113	+6 Gm. to 7 Gm.	2.31	2.08
IV	193	+7 Gm. to 8 Gm.	2.36	2.23
V	101	+8 Gm. to 9 Gm.	2.40	2.23
VI	66	+9 Gm. to 10 Gm.	2.50	2.40
VII	19	+10 Gm. to 11 Gm.	2.63	2.41
VIII	13	+11 Gm. to 12 Gm.	2.67	2.53
IX	2	+12 Gm. to 13 Gm.	2.70	2.70
X	5	+13 Gm. to 14 Gm.	2.87	2.56
XI	1	+14 Gm.	2.80	3.25

SUMMARY AND CONCLUSIONS

As a result of the investigation carried out upon commercial samples of areca, the following observations were made:

1. According to the present N. F. VII standards for weight, 98.9 per cent of the seeds examined were not official.

2. The weights varied from 1.45 to 14.57 Gm.

3. All weight samples met the N. F. VII assay requirement of not less than 0.35 per cent of ether-soluble alkaloids calculated as arecoline.

4. According to the present N. F. VII standards for measurements, 99.4 per cent of the seeds examined were not official.

5. The diameters varied from 1.60 to 2.80 cm.

6. The lengths varied from 1.30 to 3.25 cm.

7. All measurement samples when assayed, however, met the N. F. VII assay requirement of not less than 0.35 per cent

of ether-soluble alkaloids calculated as arecoline.

8. A representative sample made by mixing equal quantities of all samples came well within the N. F. VII 2.5 per cent maximum limit for total ash.

9. Samples assayed by adding the fiftieth-normal sulfuric acid directly to the flask containing the decanted 50 cc. ether solution which had been concentrated to one-third of its volume gave results which checked well within the tolerable limits and a greater number of samples could be assayed in the same length of time by this procedure.

10. A moderately coarse powder was used in the assay and gave results higher than the N. F. VII 0.35 per cent minimum limit for ether-soluble alkaloids calculated as arecoline.

11. Distilled water was used in the assay process instead of water, which is permitted in the N. F. VII assay process, for the sake of accuracy, and thereby to conform with most of the assay procedures in the N. F. VII and the U. S. P. XII.

These findings lead to the following conclusions:

1. Inasmuch as 98.9 per cent of the seeds examined did not come within the N. F. VII weight standards, yet met the N. F. VII assay requirement of not less than 0.35 per cent of ether-soluble alkaloids calculated as arecoline, it seems that the N. F. VII 5-Gm. maximum-weight limit is not necessary.

2. Since 99.4 per cent of the seeds examined did not meet the N. F. VII measurement requirements, but were found to be within the N. F. VII assay limit of not less than 0.35% of ether-soluble alkaloids calculated as arecoline, the N. F. VII maximum measurement limits seem to be unnecessary.

3. Because samples assayed by adding the fiftieth-normal sulfuric acid directly to the flask containing the decanted 50 cc. ether solution, which had been concentrated to one-third its volume, gave results comparable to those extracted in the separator, and thus allowed the extraction of many samples simultaneously, it seems that the N. F. should permit this deviation in the assay process.

4. As neither the N. F. VII nor the U. S. P. XII define a moderately fine powder and since a moderately coarse powder is defined in the U. S. P. XII (4), and this size powder gave excellent assay results, it seems that the N. F. VII should change the assay to read moderately coarse powder.

5. Inasmuch as most of the assay processes in the N. F. VII and the U. S. P. XII use distilled water, it seems that the N. F. VII should observe this precaution as necessary, also for the sake of uniformity.

In view of the findings herein presented, it is recommended that the N. F. VII Subcommittee suggest the deletion, from the monograph, in the paragraph titled "Unground Areca," of the statement, "up to 3 cm. in length and 2 cm. in diameter; each seed up to 5 Gm. in weight." Also, in the assay process change the statement, "in moderately fine powder," to read: "in moderately coarse powder." And in every instance where water is directed to be used, prefix "distilled."

A proposed monograph revision for Areca would then read as follows:

ARECA

Arecanut Betelnut

Areca is the dried ripe seed of *Areca catechu* Linné (Fam. Palmae). Areca contains not more than 2 per cent of adhering pericarp and not more than 1 per cent of other foreign organic matter and yields not less than 0.35 per cent of ether-soluble alkaloids calculated as arecoline and not more than 2.5 per cent of total ash.

Unground Areca.—Rounded-conical; externally weak reddish brown to light yellowish brown, marked with a network of paler lines, and frequently with adhering portions of silvery brittle endocarp and adhering fibers of the mesocarp at the base of the seed. Seed hard, the cut surface exhibiting a marbled appearance (ruminate endosperm) of brownish tissue alternating with whitish tissue.

Histology.—Seed coat of several rows of tangentially elongated cells with the inner walls more or less thickened; the whitish endosperm tissue cells with thick porous walls and containing oil globules and aleurone grains; the brownish perisperm tissue with thin-walled cells and delicate tracheae.

Powdered Areca.—Color weak reddish brown to light brown; odor slight; taste astringent, slightly bitter; consisting principally of fragments of the endosperm tissue with porous reserve-cellulose walls; irregularly thickened stone cells of the seed coat; a few aleurone grains up to 40 microns in diameter and a few oil globules; starch absent, and tracheal tubes few.

Assay.—Place 8 Gm. of areca, in moderately *coarse* powder and accurately weighed, into a suitable flask, add 80 cc. of ether, shake well, add 4 cc. of ammonia T. S., and shake for ten minutes. Add 10 Gm. of anhydrous sodium sulfate and shake for five minutes. Allow to settle and decant the ether into another flask. Add 0.5 Gm. of talc to the decanted ether solution and shake for three minutes, then add 2.5 cc. of *distilled* water and shake for three minutes more. Allow to stand until clear, decant 50 cc. of the ether, equivalent to 5 Gm. of areca, and distill off about two-thirds of the ether. Extract the remaining ether in a separator, or *in the distilling flask* with 15 cc. of fiftieth-normal sulfuric acid and then with three portions of *distilled* water, using 5 cc. each time. To the combined acid and washings, add methyl red T. S. and titrate the excess acid with fiftieth-normal sodium hydroxide solution. Each cc.

of fiftieth-normal sulfuric acid is equivalent to 0.0031 Gm. of arecoline.

AVERAGE DOSE—

	Metric	Apothecaries
Dogs	2–4 Gm.	30–60 gr.
Sheep	4–8 Gm.	1– 3 dr.

Based on the weight of the animal.

REFERENCES

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(2) *Bull. National Formulary Subcommittee No. 1*, June (1943), 325.
(3) "United States Pharmacopœia XII", Mack Printing Company, Easton, Pa., 1942, p. 628.
(4) *Ibid.*, p. 583.

The Leucocyte Response to Pyrogen in Rabbits*

By F. J. Bandelin†‡

The presence of thermogenic substances in distilled waters has been known almost as long as intravenous therapy has been in general use.

As early as 1911 Wechselmann (1) noticed an elevation in temperature accompanying the intravenous injections of arsphenamine solutions and demonstrated that the reaction could be eliminated by the use of germ-free distilled water. About the same time Hort and Penfold (2) found that water distilled from a Jena glass retort, if used immediately for injection, produced no fever. If the water was allowed to stand for forty-eight hours, however, it became pyrogenic. The comprehensive investigations of Seibert (3) ruled out a number of factors such as dissolved salts and gases and the *p*H of the water, among other factors, as possible thermogens and showed that the fever-producing substances were of bacterial origin (3, 4).

Bourn and Seibert (5) identified two groups of river bacteria which were capable of causing fever. Rademaker (6, 7) confirmed the work of Seibert and found that, in the distillation of water, the first fraction produced severe reactions and the middle fraction gave no noticeable physiological response, while the residue produced a sustained fever. He also set forth a number of rules to be observed in the preparation of pyrogen-free water. Perkins (8) and Walter (9) having prepared water according to the specifications of Rademaker stated it to be free from reactions.

Co Tui and his co-workers (10, 11, 12, 13, 14) described a method for removing pyrogen from solutions by adsorptive filtration and estimated from this work that the pyrogenic agent is of a size from 50 mμ to 1 micron. During this investigation they also found a leucopenia in dogs resulting within forty-five minutes after the injection of pyrogenic material and suggested that a drop of 5000 cells in dogs be regarded as a definite leucopenia.

Chapman (15), in a publication appearing while this paper was in preparation, demonstrated a marked leucopenia accompanying the fever reaction in rabbits after the injection of pyrogen and suggests that a drop of 4000 leucocytes per cubic millimeter from the observed normal leucocyte count in rabbits may be regarded as indicative of pyrogen in solution injected intravenously.

Welch, *et al.* (16), have suggested a method of preparing and testing pyrogens by the thermogenic response in rabbits and have shown pyrogen to be free of antigenicity. McClosky, *et al.* (17), have on this basis evolved a test which is the basis for the U. S. P. XII method of pyrogen determination (18).

Interest in methods of pyrogen determination in distilled water intended for injection has recently been intensified by the inclusion of the pyrogen test in the U. S. P. XII.

Because of the variability of the fever reaction in rabbits, after the injections of pyrogen, this investigation was undertaken to find a more reliable and possibly a more sensitive method for pyrogen detection.

Preliminary investigation of the variation in the white blood cell count in rabbits following the injection of pyrogen solutions, indicated that this reaction may be sufficiently sensitive to be considered as a method for pyrogen determination. This approach has yielded gratifying results.

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‡ The author is indebted to Dr. L. J. Klotz, Dean, Cincinnati College of Pharmacy, for his valuable suggestions and criticisms, and to Mr. Keith Overman for his technical assistance.

In our work we have attempted to show that the leucocyte count in rabbits may be a more rapid and more sensitive method for pyrogen determination than the present official method.

In order to evaluate the leucocyte response to pyrogen, we have attempted to correlate the change in the leucocyte count with relative elevation in temperature after injection of varying dilutions of a standard pyrogen solution as well as tap and river water.

EXPERIMENTAL

Preparation of Pyrogen.—A stock pyrogen solution was prepared by the method of Welch, *et al.* (16). A culture of *Pseudonomas aeruginosa*[1] was grown in F. D. A. broth (19) for twenty-four hours, then transferred to F. D. A. agar (19) in Roux bottles and incubated for forty-eight hours. The resulting growth in the form of a thick pellicle was washed from the agar with distilled water and pooled aseptically in sterile flasks, then filtered through gauze and cotton. This liquid was then shaken in a bottle with glass beads on a mechanical shaker for five hours. It was then incubated at 37° C. for six days and then centrifuged at 4000 r. p. m. The supernatant liquid was filtered under aseptic conditions through a Berkefeld filter. Sterility tests were conducted by the U. S. P. XII method (20) and found to be negative. The nitrogen content was determined by micro-Kjeldahl and the solution diluted with freshly distilled sterile water containing 0.85 per cent NaCl so that each 100 cc. contained 5 mg. of total nitrogen (0.05 mg. per cc.).

Experimental Procedure.—All rabbits used were eight to twelve months of age and weighed more than 2.0 Kg. They were kept at uniform temperature and on a uniform diet in individual cages. Four rectal temperatures were taken on all rabbits at two-hour intervals on the two days preceding the test. On the test day two rectal temperatures at 2 hour intervals were taken. If these temperatures did not vary more than 1.0° F. the rabbit was considered well and the last temperature taken was regarded as normal. The volume of all test solutions was adjusted with freshly distilled sterile water so that the rabbit received 10 cc. per Kg. of body weight. The injection was made into the marginal ear vein, using aseptic technic, fifteen minutes after the final normal temperature reading. The rate of injection was adjusted so that the solution entered the vein at a rate not exceeding 5 cc. per minute. Care was exercised to avoid exciting the rabbits before or during the test. Leucocyte counts were made on two consecutive hours just prior to the test to determine the individual normal. If these counts did not vary more than 2000 cells, the rabbit was considered eligible for the test. The last W. B. C. count was taken as the normal count. Temperature readings were taken every hour for four hours after injection and the leucocyte count was observed at the same time. The leucocyte count was further observed at varying intervals of not more than two hours each for twenty-four hours. Rabbits were given water ad libitum after injection but food was withheld for twenty-four hours.

[1] Obtained from the American Type Culture Collection. Georgetown University, Washington, D. C.

TABLE I.—COMPARISON OF LEUCOCYTE AND FEVER RESPONSE IN RABBITS TO VARIOUS DILUTIONS OF STANDARD PYROGEN SOLUTION

Rabbit No.	Dilution	Change in Temperature °F.	Initial W. B. C. Decrease × 1000	Secondary W. B. C. Increase × 1000
1	1:3500	+4.2	− 9.6	+14.9
2		+3.1	− 7.9	+11.7
3		+2.2	− 8.8	+19.8
4		+4.0	−10.2	+15.6
5		+3.8	− 7.7	+ 6.3
6	1:4000	+1.8	− 8.6	+14.1
7		+2.3	− 6.6	+11.4
8		+1.9	− 7.9	+12.9
9		+2.8	− 9.4	+ 9.9
10		+2.2	− 6.3	+12.2
11	1:4500	+1.2	− 7.2	+11.4
12		+0.9	− 9.4	+16.3
13		+1.3	− 4.4	+13.9
14		+0.8	− 8.3	+ 9.7
15		+1.6	− 7.3	+10.4
16	1:5000	+0.6	− 6.4	+ 9.6
17		+0.9	− 5.1	+14.3
18		+0.8	− 8.3	+11.9
19		+0.9	− 6.6	+ 8.3
20		+1.6	− 8.8	+12.2
21	1:5500	+0.2	−11.4	+ 9.3
22		−0.3	− 7.4	+11.4
23		+0.4	− 6.6	+17.1
24		+0.1	− 4.3	+ 9.9
25		+0.8	− 7.7	+11.4
26	1:6000	+0.3	− 8.8	+ 9.4
27		−0.4	− 4.4	+ 6.7
28		−0.1	− 6.5	+ 9.6
29		+0.8	− 5.3	+10.0
30		+0.3	− 4.3	+ 9.3
31	1:6500	+0.2	− 3.3	+ 6.6
32		+0.3	− 4.6	+ 1.4
33		+0.2	− 2.9	+ 6.7
34		0.0	− 5.6	+ 8.4
35		+0.5	− 1.1	+ 2.3
36	1:7000	+0.1	− 1.6	+ 3.0
37		−0.3	− 2.4	+ 2.5
38		−0.1	+ 3.1	+ 4.2
39		+0.3	0.0	− 2.6
40		+0.3	+ 0.3	+ 5.2

Preliminary investigations were conducted by injecting increasing dilutions of stock pyrogen solution in sterile distilled water (10 cc. per Kg.) into rabbits. Temperature and leucocyte reactions were noted. This was done to establish the approximate level at which the dilution produced a diminution either in the magnitude of the temperature rise or of the leucocyte response. This appeared to occur at a dilution between 1:3500 and 1:4000. At this level, a decrease in the fever response was noted and the temperature rise became less marked as the dilution increased. It disappeared or fell within normal variation at a dilution between 1:4500 and 1:5000. The leucocyte reaction persisted however at greater dilutions and disappeared finally at 1:6500.

A series of 40 rabbits (nos. 1–40) were then injected with the increasing dilutions of the stock

pyrogen solution at 1:500 increments from 1:3500 to 1:7000. At this latter dilution, the leucocyte responses were of a magnitude encountered after the injection of sterile distilled water. Five rabbits were injected with the dilutions at each level, the dilution increasing by 1:500 at each gradation. Temperature and leucocyte counts were observed as described above.

A second series of rabbits (nos. 41–55) were injected with sterile distilled water, tap water and river water, after which temperatures and leucocyte reactions were noted. Rabbits 41–45 were injected with sterile distilled water, rabbits 46–50 with tap water and 51–55 with river water. This was done to determine if the leucocyte response is caused by common water contaminants and not specific for the particular pyrogen under investigation.

Normal Leucocyte Count in Rabbits.—To establish the normal leucocyte count in rabbits, counts were made at frequent intervals throughout the day on a number of rabbits during a period of several weeks and the variation in cell count noted.

We found a variation of from 5000–11,500 leucocytes per cubic millimeter as compared to 3000–12,000 reported by Kolmer and Boerner (21) and from 8000–13,000 as given by Gradwohl (22). The "hourly rhythm" or gradual leucocyte increase noted in man throughout the day could not be established for rabbits. The maximum variation found in any one-hour period, however, did not exceed 2000.

The white blood cell count made immediately before injection was regarded as a normal count.

DISCUSSION

Within thirty minutes after injection of reactive solutions, a decrease in the white blood cell count occurred coincident with the temperature rise. The temperature usually returned to normal in about five to six hours after having attained a peak in from three to four hours. It was noted that the white cell increase following the leucopenia did not stop at the normal white cell count but continued to rise producing a marked white blood cell increase. The peak of this leucocytosis usually occurred between eight and sixteen hours after injection and required about eighteen to twenty-four hours to return to normal.

The leucopenia or leucocytosis expressed in the tables does not necessarily indicate the lowest or highest white cell count since these may have occurred between observations in some cases.

Both the fever and leucocyte response became progressively less marked as the dilution of the pyrogen solution increased. The magnitude of the fever response began to decrease at a dilution of 1:3500 and continued to decrease until it fell within normal limits, as established for the test at 1:5000. One exception was encountered in the rather extreme response of rabbit no. 20.

The leucocyte response persisted, however, and did not fall to within normal variations until a dilution of 1:6500. Some response was still evident at this point since a leucopenia of 4000 cells below the observed normal count was regarded as evidence of pyrogen. This was an arbitrary figure arrived at by doubling the maximum observed hourly white blood cell variation. Rabbits 32 and 34 showed white cell decreases of 4600 and 5600 respectively. At a dilution of 1:7000, all counts were within normal variation.

Erratic fever and leucocyte responses were commonly encountered in the animals and these were considered ineligible for the test.

TABLE II.—LEUCOCYTE AND FEVER RESPONSE IN RABBITS TO DISTILLED WATER, TAP WATER AND RIVER WATER

Rabbit No.	Cc. per Kg.	Change in Temperature ° F.	Initial W. B. C. Decrease × 1000	Secondary W. B. C. Increase × 1000
		Distilled Water		
41	10 cc.	−0.3	− 0.4	+ 2.9
42	10 cc.	−0.1	0.0	− 0.4
43	10 cc.	+0.4	− 0.4	+ 4.1
44	10 cc.	+0.25	− 0.3	− 3.7
45	10 cc.	+0.2	+ 0.6	+ 1.6
		Tap Water		
46	10 cc.	+2.8	− 5.5	+17.6
47	10 cc.	+2.4	− 7.2	+15.4
48	10 cc.	+3.1	− 6.9	+11.2
49	10 cc.	+2.9	− 9.1	+ 9.2
50	10 cc.	+2.4	− 6.3	+11.9
		River Water		
51	10 cc.	+3.8	− 6.3	+14.7
52	10 cc.	+4.1	− 9.2	+23.2
53	10 cc.	+2.2	−10.1	+14.4
54	10 cc.	+3.3	− 7.2	+10.9
55	10 cc.	+3.6	− 9.1	+14.6

Although there is no evidence to indicate that the actual pyrogenic material is a nitrogenous compound, the nitrogen content was used in standardizing the solution since it has been demonstrated (16) that fairly consistent fever response may be expected from solutions of identical nitrogen content. We observed, however, slightly different fever and leucocyte extinction points, on dilution, with similarly prepared solutions of the same nitrogen content. The ratio of dilution at which both responses disappear remained about the same however.

Rabbits injected with freshly distilled sterile water gave no noticeable fever or leucocyte reaction. Injections of tap water and river water, however, produced a marked fever and leucocyte reaction indicating that common water contaminants produce leucocyte response as well as fever.

From these studies it seems the white blood cell response to the injection of pyrogen is a more sensitive indication of the presence of pyrogen than the temperature rise since a dilution which is no longer thermogenic gives a leucocyte response in all cases observed. Either the leucopenia observed sixty to ninety minutes after injection or the subsequent leucocytosis may be regarded as evidence of pyrogenicity. It is significant that the leucopenia and the leucocytosis disappear at the same concentration of pyrogen. The leucopenia is usually represented by a decrease of 4000–9000 cells and the leucocytosis by an increase of 10,000 cells or more. Leucocyte counts as high as 32,000 cells per cubic millimeter have been observed.

SUMMARY AND CONCLUSIONS

1. The intravenous injection of pyrogen solutions produce, in addition to the fever response, a primary leucopenia followed by a secondary leucocytosis.

2. The leucopenia represented by a decrease of 4000–9000 white cells usually occurs sixty to ninety minutes after injection and is followed by a leucocytosis represented by a white blood cell increase of 10,000 cells or more.

3. The fever and leucocyte response became less marked with progressive dilutions of pyrogen with the fever response disappearing before the leucocyte reaction.

4. The leucocyte response in rabbits seems to be a more sensitive and rapid indication of pyrogen than the fever reaction. The initial leucopenia which is evident in a blood sample taken one hour after injection can be regarded as definite evidence of pyrogenicity. The secondary leucocytosis occurring later may be used to corroborate the primary leucopenia.

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Some Physical and Chemical Properties of Commercial Racemic Menthol*

By Kenneth L. Waters and George D. Beal†

The first partly successful attempts to synthesize menthol were recorded by Brunel (1) in 1905. On hydrogenating thymol he obtained a mixture of menthol, neo-menthol, menthone and methylcyclohexanol. Pickard and Littlebury (2) succeeded in isolating racemic (*dl*) menthol about 1912. Since that time the eight stereoisomers and their four racemic mixtures have been obtained. Bliss and Glass (3), and Hall, Holcomb and Griffin (4) in this country, and Huggett (5, 6) in England have adequately described the separation, preparation and properties of these isomers. Table I is a condensation of Huggett's tabulation of the physical constants of the menthol isomers. Commercial processes have been developed whereby a high grade of racemic menthol can be produced and Wagner (7) has summarized a number of patents outlining these processes.

When the British and the Germans found their supplies of natural *l*-menthol jeopardized, their respective pharmacopœias (8, 9) gave official status to racemic menthol. The United States Pharmacopœia (10) has considered the racemic form of menthol worthy of recognition. Prior to the preparation of the monograph, this laboratory made an investigation of a number of samples of racemic menthol. Various individuals submitted these samples and stated that they were commercially acceptable racemic menthols. Examination was also made of a number of samples consisting of isomeric menthol mixtures and of menthol by-products of synthetic processes. No attempt was made to purify any of the samples; accordingly, constants and other characteristics reported in this work must be considered as those of commercial, not highly purified, products. During the course of the investigation critical consideration was given to all tests listed by the British and German Pharmacopœias. Some of these tests were found to be excellent and are therefore included in the new U. S. P. monograph.

TABLE I.—PHYSICAL CONSTANTS[a] OF MENTHOL ISOMERS

Stereoisomer	Melting Point, ° C.	Boiling Point at 760 Mm. ° C.	$[\alpha]_D^{25}$ in 20% Ethyl Alcohol
l-Menthol	43.0	216.5	−50.0
dl-Menthol	38.0[b]	216.5	0
d-neo-Menthol	−15.0	211.7	+20.0
dl-neo-Menthol	52.0	211.7	0
d-iso-Menthol	82.5	218.6	+26.0
dl-iso-Menthol	53.5	218.6	0
d-neo-iso-Menthol	− 8.0	214.6	+ 2.0
dl-neo-iso-Menthol	13.5	214.6	0

[a] From Huggett (5).
[b] Bliss and Glass (3) give 34.0 as the melting point of *dl*-menthol.
("The dextro- and laevo- forms of the same isomer have been found to have the same or very similar physical properties, and in compiling Table I only one active and the racemic form of each isomer was included.")

Generally, the melting point is one of the first criterions considered when it is desired to check the identity and purity of an organic compound. Unfortunately the melting point of racemic menthol is not too well defined. Most of the samples melted slowly over a range of one and a half degrees. A possible explanation might be that the commercial samples were impure; however, a melting range from 28° to 38° C., depending upon the conditions of the experiment, has been recorded (5), and attributed to the fact that there are two crystalline modifications of racemic menthol, melting at 28° and 38° C., respectively. Most literature references give the melting point of racemic menthol as 34.0° C. The British Pharmacopœia has set the limits for acceptable racemic menthol as 32.5° to 34.0° C. The German Pharmacopœia gives limits from 32° to 36° C. In view of the adoption of these limits by official compendia it might reasonably be expected that the melting point would be close to 34° C. when determined by the usual methods. By following the United States Pharmacopœia procedure for the determination of the melting points of Class I compounds (11), the authors have been able to obtain consistent results. Unfortunately, when other individuals were asked to check the melting points of some of the samples of racemic menthol, there was a variation in results.

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Experiments indicate that the rate of heating is not a critical factor in the melting-point determination. On the other hand, the method of preparing the sample for the determination of the melting point definitely affects the melting point.

A study of the effect of humidity on the melting point was made. Indications are that the melting point of pure racemic menthol is not appreciably affected by humidity. Accordingly, it must be assumed that racemic menthol is not very hygroscopic. It has previously been reported (6) that racemic menthol will melt on prolonged exposure at 30–31° C. Investigation has shown that in a constant temperature room at 28° C. there is a tendency for racemic menthol to liquefy at relatively high humidities. This tendency is not so pronounced at low humidities. Traces of water vapor along with traces of other impurities probably affect the equilibrium which exists between the two crystalline forms.

TABLE II.—MELTING POINTS OF RACEMIC MENTHOL

Sample No.	Melting Points, ° C.			
	Authors	"A"	"B"	"C"
4	34.4	33.8	33.7	34.2
2	34.6	35.4	33.7	35.0

of samples of racemic menthol containing small amounts of other isomers indicates this is a rather accurate criterion of purity. Huggett (5) and Serini (12) have endorsed this phenomenon as a method of distinguishing isomer-free racemic menthol from other menthols. Both the British and the German Pharmacopœias have included the setting or congealing point as a characteristic test.

The German Pharmacopœia employs 0.01 per cent potassium permanganate for the detection of "Foreign organic substances." A study of the sensitivity of this test toward possible contaminants of racemic menthol has been made. By using a standardized procedure it was found possible to detect as low as 0.1 per cent of thymol or 0.01 per cent of *m*-cresol. Indications are that easily oxidizable oil residues may also be detected by the use of potassium permanganate. A racemic menthol sample containing 0.5 per cent of U. S. P. Oil of Peppermint caused the disappearance of the permanganate color in about eight minutes. Some forty samples of various kinds of menthol were tested for easily oxidizable substances.

It is frequently alleged that racemic menthol develops an "off" odor on storage. Our experience has been that a sample of racemic menthol which has been on hand for two years still possesses a pleasant odor and

TABLE III.—MELTING POINTS (° C.) OF RACEMIC MENTHOL AT VARIOUS HUMIDITIES IN CONSTANT TEMPERATURE ROOMS AT 21° C. AND 28° C.

Approximate Rel. Humidity: Sample No.	Room Temp.	100%	80%	60%	40%	20%	0%
1	21°	35.5	35.0	35.0	35.0	35.2	35.2
	28°	Paste[a]	34.8	34.8	35.0	35.0	35.0
2	21°	33.8	34.2	34.2	34.2	34.2	34.6
	28°	Liquid[a]	Paste[b]	34.2	34.2	34.0	34.6
3	21°	32.6	34.0	34.0	34.0	34.2	34.2
	28°	Liquid[a]	Paste[c]	34.0	34.0	34.0	34.0
4	21°	34.0	34.0[e]	34.0[e]	34.2[e]	34.2	34.4
	28°	Liquid[a]	Paste[d]	34.0	33.8	34.0	34.4

[a] Transferred to 21° C. room for twenty-four hours in same desiccator, no crystallization occurred. Removed to sulfuric acid desiccator for twenty-four hours. Melting point returned to normal.
[b] Paste-like, which completely liquefied at 33.8° C.
[c] Paste-like, which completely liquefied at 32.4° C.
[d] Paste-like, which completely liquefied at 33.6° C.
[e] Exposure continued for one month with no change in melting point or general appearance.

Although the two crystalline modifications of racemic menthol may lead to an ill-defined melting point, they produce a phenomenon which is very characteristic and serves excellently as a test for both identity and purity. When racemic menthol is melted and cooled a definite setting point is observed at 27–28° C.; after stirring for several minutes the temperature is seen to rise sharply to about 31° C. These points are very sharp and characteristic. Examination meets all the requirements of the new monograph. One sample was subjected to the drastic storage conditions of being alternately melted and crystallized for a period of six months. This sample showed no change in physical or chemical properties.

EXPERIMENTAL

Melting Point.—Consistent values for the melting point were obtained by followng the U. S. P. XII procedure for Class I compounds (11). Samples

were chilled, pulverized in a chilled mortar, dried over sulfuric acid for twenty-four hours at room temperature, and then introduced into capillary tubes. Heating was at the rate of three degrees per minute until the first break against the side of the tube occurred, then at a rate of one-half degree per minute until the sample was completely liquefied. Most samples tested gave a range of one and a half degrees from the first break to complete liquefaction.

Two samples of racemic menthol were of particular interest because they were submitted to the laboratory with the statement that the melting point was under 32.5° C., but the authors found the melting points to be somewhat higher. Because of this discrepancy, three individuals, "A," "B" and "C," were asked to check the melting point of these samples, using the U. S. P. XII procedure. These results are summarized in Table II.

"A" followed the same procedure as did the authors, but with the omission both of chilling the sample and of pulverizing in a chilled mortar.

"B" filled the capillary tubes with molten menthol and allowed the filled tubes to stand over sulfuric acid in a desiccator at approximately 5° C. overnight. He also reported that if the capillary tubes were filled with molten menthol and the melting point determined shortly after crystallization took place, a melting point of 28.5° C. was obtained for both samples.

TABLE IV.—REACTION OF MENTHOL SAMPLES TOWARD 0.01% POTASSIUM PERMANAGANATE

	Source	Melting Point, ° C.	$[\alpha]_D^{25}$	Color After Time Intervals, Min. 1	2	3	4	5	10
1	*m*-Cresol	35.0	0	P	P	P	P	P	P
2	Turpentine	34.6	0	P	P	P	P	BP	BR
3	Turpentine	34.0	0	P	P	P	P	BP	BR
4	*p*-Cymene	34.4	0	P	P	P	P	P	P
5	*p*-Cymene	33.4	− 0.5	P	P	P	BP	BR	BY
6	Unknown	33.8	− 0.5	P	P	P	P	P	P
7	Unknown	33.8	− 0.5	P	P	P	P	P	P
8	By-Product of *l*-Menthol from Citronella	35.8	−32.0	P	P	P	P	P	P
9	By-Product of *l*-Menthol from Citronella	35.0	−30.0	P	P	P	P	P	P
10	By-Product of *l*-Menthol from Citronella	30.0	− 5.0	P	DY	X	X	X	X
11	By-Product of *l*-Menthol from Citronella	30.0	− 5.0	P	DY	X	X	X	X
12	By-Product of *l*-Menthol from Citronella	Liquid	−24.0	BR	DY	X	X	X	X
13	By-Product of *l*-Menthol from Oil of Peppermint	39.0	−40.0	P	P	P	P	P	P
14	By-Product of *l*-Menthol from Oil of Peppermint	36.0	−42.5	P	DY	X	X	X	X
15	By-Product of *l*-Menthol from Oil of Peppermint	28.0	−41.0	DY	X	X	X	X	X
16	*p*-Cymene	28.0	0	P	P	P	BP	BY	BY
17	Thymol	28.0	0	P	P	P	P	P	P
18	Thymol	Liquid	0	P	BP	BR	BY	X	X
19	Thymol	Liquid	0	P	P	P	P	P	BP
20	Thymol	Liquid	− 0.5	P	P	P	P	P	BP
21	Thymol	Liquid	0	P	P	P	P	P	BP
22	Thymol	Liquid	0	P	BP	BR	BR	BR	DY
23	Unknown	32	−21.5	P	P	P	BP	BP	BR
24	Unknown	Liquid	−12.5	BP	DY	X	X	X	X
25	Unknown	Liquid	0	DY	X	X	X	X	X
26	Unknown	Liquid	− 1.5	BP	DY	X	X	X	X
27	Labeled U. S. P.	42.0	−49.0	P	P	P	BP	BP	BR
28	Labeled U. S. P.	43.0	−49.0	P	P	P	BP	BP	BR
29	Labeled U. S. P.	42.8	−49.0	P	P	P	P	P	P
30	Labeled U. S. P.	42.8	−50.0	P	P	P	P	BP	BR
31	American Mentha Piperita	40.0	−47.5	BR	DY	X	X	X	X
32	Japanese Mentha Arvensis	43.0	−49.5	P	BP	BR	BR	BY	DY
33	Brazilian Mentha Arvensis	42.8	−49.5	P	P	BP	BR	BY	DY
34	Chinese Mentha Arvensis	42.6	−50.0	P	P	BP	BR	BY	DY
35	American Mentha Arvensis	42.6	−48.5	BP	BY	DY	X	X	X
36	Synth. from Java Citronella	42.6	−50.0	P	P	P	P	P	P
37	*l*-Menthol + *dl*-Menthol	..	..	P	P	P	P	P	P
38	*dl*-Menthol + 1% Thymol	..	..	DY	X	X	X	X	X
39	*dl*-Menthol + 0.1% Thymol	..	..	P	BP	BR	BR	DY	X
40	*dl*-Menthol + 0.01% Thymol	..	..	P	P	P	BP	BP	BR
41	*dl*-Menthol 1% *m*-Cresol	..	..	DY	X	X	X	X	X
42	*dl*-Menthol + 0.1% *m*-Cresol	..	..	DY	X	X	X	X	X
43	*dl*-Menthol + 0.01% *m*-Cresol	..	..	P	P	BP	BR	BY	DY
44	*dl* Neo-Menthol	50.0	..	P	P	P	P	P	P

NOTE: P = Purple, BP = Brownish Purple, BR = Brownish Red, BY = Brown Yellow, DY = Dirty Yellow, X = No Observation.

"C" followed the U. S. P. procedure as outlined by the authors. He also reported that the samples melted over a range of four degrees.

From the observation made by "B," it is obvious that the method of preparing the sample for the determination of the melting point has a definite effect on the value obtained. In order to obtain a clear insight into just how much the method of preparation did affect the melting point, a number of capillary tubes were filled with racemic menthol, melted, then congealed by dipping the tube in cold water, and the melting point determined as soon as solidification was apparently complete. In this manner a melting point between 28° and 30° C. could be obtained for a racemic menthol which had a melting point of 34° C. as determined according to the U. S. P. procedure. When the same capillary tubes in which this melting point had been determined were allowed to stand for periods of one-half hour to eighteen hours at 5° C., it was observed that the melting point returned slowly to its original value, namely, 34° C. This phenomenon is, no doubt, a verification of the fact that racemic menthol may exist in different crystalline modifications, the lower-melting form crystallizing out first and slowly attaining equilibrium with the higher-melting form.

In order to determine if the rate of heating is a critical factor in the determination of the melting point a number of melting points were determined according to the authors' description of the U. S. P. procedure but with rates of heating varying from one degree per minute to four degrees per minute. In each instance, when the first break occurred the rate of heating was slowed to one-half degree per minute. The melting-point values obtained with the different rates of heating did not show appreciable variation.

A study of the effect of humidity on the melting point of racemic menthol was made by placing powdered racemic menthol samples on watch glasses in desiccators containing saturated salt solutions. Solutions were chosen which would give approximate relative humidities of 100, 80, 60, 40 and 20%. Saturated solutions of calcium sulfate, ammonium chloride, sodium bromide, zinc nitrate and potassium acetate, respectively, were selected as approximating these humidities (13). Concentrated sulfuric acid was used to produce low humidity. The constant humidity desiccators were placed in constant temperature rooms at 21° and 28° C., respectively, and the melting point of the racemic menthols determined after forty-eight hours.

Table III shows the melting-point values obtained at the various humidities in the two constant temperature rooms. It was found that, at 28° C., certain racemic menthols liquefied at high humidities. However, samples of the *same* racemic menthol when treated in the same manner in a constant temperature room at 21° C. showed little effect on the melting point. From the values obtained at 21° C., it would seem that racemic menthol is not appreciably hygroscopic. However, the humidity must have some effect on the product as indicated by the fact that at the same temperature, (i. e., 28°) samples exposed to relative humidities in excess of 80% liquefied, whereas those exposed to a relative humidity of 60% showed no tendency to liquefy. It is interesting to note that the samples which liquefied at high humidities in the 28° C. constant temperature room failed to crystallize when transferred to the 21° C. constant temperature room and to the same high humidities; however, when transferred to the desiccator containing sulfuric acid they crystallized and the melting point returned to the normal value. Water vapor probably affects the equilibrium between the two crystalline forms of racemic menthol, the effect being more pronounced in the presence of traces of impurities.

Congealing Point.—The following procedure was found to give consistent results in the determination of the congealing point or points:

Place 10 Gm. of racemic menthol in a dry test tube of from 18 to 20 mm. internal diameter, and melt the contents at about 40° C. Suspend the test tube in water having a temperature of 23–25° C. and stir the contents of the tube constantly with a thermometer, keeping the bulb of the thermometer immersed in the liquid.

Most of the racemic menthols tested super-cooled to about 25° C.; then the temperature rose to a constant value of about 27.5° C. and the sample remained in a semisolid condition at this temperature for about three to five minutes, then quickly rose to about 31° C., where it congealed to a solid mass.

Two samples of racemic menthol which contained 1% and 3%, respectively, of racemic neomenthol were prepared. The original racemic menthol had an initial congealing point of 28° C. and showed a sharp rise to 31° C. When adulterated with 1% of this neomenthol the initial congealing point of the mixture was 27° C. and rose to only 29.5° C. on continued stirring. When adulterated with 3% of neomenthol the initial congealing point of the mixture was 25.5° C. and this rose to 27.5° C. on continued stirring.

Easily Oxidizable Substances.—Some forty samples of menthols were tested for their reducing action on potassium permanganate. These samples were from various sources; some were racemic menthols, some were *l*-menthols, others were by-products of various processes for manufacturing menthol. The source (when known), the melting point, optical rotation and action toward 0.01% potassium permanganate of these various menthols are listed in Table IV. The following procedure was found to give consistent results in the detection of easily oxidizable substances:

Place 0.5 Gm. of menthol in a clean test tube, add 10 cc. of 0.01% potassium permanganate (freshly prepared), and place the test tube in a beaker of water having a temperature between 45° and 50° C. Remove the tube from the bath at intervals of thirty seconds and mix quickly by shaking.

Oxidizable material present in the menthol reduces the permanganate from its original clear purple color through successive turbid colors to a dirty yellow with precipitation of manganese oxides. In Table IV these changes are described as purple, brownish purple, brownish red, brownish yellow and dirty yellow. Brownish red may be construed as "still having some of the original permanganate color remaining;" however, some individuals may judge that there is no such color remaining.

It may be noted from Table IV that the racemic menthols with melting points of 33° to 35° C. do not remove the color of permanganate solution rapidly. One also notes that some of the *l*-menthols reduce permanganate rather quickly. This is probably due to the presence of residual oils. In order to test this hypothesis, racemic menthol samples which contained 0.25, 0.5 and 1.0% of Oil of Peppermint were prepared. These samples were tested for their reducing action on permanaganate. The racemic menthol adulterated with 1% of peppermint oil reduced the permanganate solution to a dirty yellow color in about four minutes. The racemic menthol adulterated with 0.5% peppermint oil required about eight minutes. That adulterated with 0.25% peppermint oil failed to remove the permanganate color completely after a treatment of about ten minutes.

Stability of Racemic Menthol.—One sample of racemic menthol which was stored under laboratory

conditions for two years had a pleasant odor and passed all the tests of the new U. S. P. monograph. Four samples showed no change in melting point or in reaction toward potassium permanganate after four months' storage. One sample was subjected to rather drastic storage conditions over a period of six months; it was placed in a clear glass jar closed with a screw top and stored near a radiator where the temperature varied from 20° to 40° C. It is estimated that in the six months' period this sample liquefied and recrystallized no less than a hundred times. At the end of this period determinations of melting point and optical activity were repeated. No change in either of these constants was noted. The action toward 0.01% potassium permanganate, as well as other tests of the new U. S. P. monograph, showed no change. Another sample, when exposed to relative humidities of 80, 60, 40% in a constant temperature room at 21° C. for a period of one month, showed no change in general appearance or in melting point. A number of samples were stored in a constant temperature room at 21° C. for two months. These samples showed no change; the powdered crystalline products had not fused.

SUMMARY

1. The melting point of racemic menthol is not an ideal criterion of the purity of the product.

2. The congealing point is considered an excellent criterion of the purity of racemic menthol.

3. A standardized test for the detection of easily oxidizable substances which are present in racemic menthol is presented in this report.

4. Racemic menthol is a stable organic compound.

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Glycol Esters in Ointment Bases*

By George W. Fiero† and Maynard W. Dutcher

Polyhydric alcohol esters, particularly glyceryl monostearate and diethylene glycol monostearate, have been widely used in cosmetics and are being prescribed in ointment bases by many leading dermatologists. The latter has been suggested for use in coal-tar ointments (1). Since many glycol esters are now available in commercial and semi-commercial quantities, it is the purpose of this study to determine their relative value as ointment base constituents.

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EXPERIMENTAL

Glycol Eesters.—In this study, the formulas used are those which have been recommended as ointment bases and which originally contained either glyceryl monostearate or diethylene glycol monostearate. Using these formulas as a base, the various glycol esters were substituted for the one in the original base. Of course, the resulting product may differ in solidity since often the quantity is such that the consistency of the resulting ointment base is stiffened by the glycol ester if it is a solid, or is softened if it is a liquid. The characteristics of the glycol esters employed appear in Table I.

Nonemulsified Ointment Bases.—Two basic formulas for nonemulsified hydrophilic ointments were employed. The first (2) utilizes sulfated hydrogenated castor oil[1] which possessed the following characteristics:

[1] Courtesy National Oil Products Co., Harrison, N. J.

TABLE I.—CHARACTERISTICS OF THE POLYHYDRIC ALCOHOL ESTERS[a]

Ester	Consistency	Color	*p*H[b]	M. P.	Solubility (Hot) Water	Solubility (Hot) Petrolatum
"Butyl Cellosolve" Laurate	Oil	Dark red	...	<0	Insoluble	Soluble
"Butyl Cellosolve" Palmitate	Semisolid	Dark amber	...	44	Insoluble	Soluble
"Carbitol" Laurate	Semisolid	Orange	...	19	Insoluble	Soluble
"Carbitol" Ricinoleate	Oil	Dark red	...	<0	Insoluble	Partly soluble
"Carbitol" Stearate	Paste	Tan	...	52	Insoluble	Soluble
"Cellosolve" Laurate	Oil	Dark brown	...	<0	Insoluble	Soluble
"Cellosolve" Ricinoleate	Oil	Dark red	...	0	Insoluble	Soluble
"Cellosolve" Stearate	Oil	Dark brown	...	16	Insoluble	Soluble
Diethylene Glycol Monolaurate	Oil	Straw	9.1	10	Dispersible	Soluble
Diethylene Glycol Dilaurate	Oil	Dark amber	...	21	Insoluble	Soluble
Diethylene Glycol Mono-oleate	Oil	Dark red	9.6	<0	Dispersible	Soluble
Diethylene Glycol Glyceryl Mono-oleate	Oil	Dark red	9.5[b]	18	Dispersible	Soluble
Diethylene Glycol Monopalmitate	Wax	Yellow	9.4[b]	49	Dispersible	Partly soluble
Diethylene Glycol Palmitate Stearate	Soft wax	Tan	6.6[b]	43	Dispersible	Soluble
Diethylene Glycol Monoricinoleate "A"	Oil	Red	...	<−50	Insoluble	Soluble
Diethylene Glycol Monoricinoleate "B"	Oil	Red	...	<0	Insoluble	Soluble
Diethylene Glycol Glyceryl Mono-rincinoleate	Oil	Dark orange	9.5	<−65	Dispersible	Partly soluble
Diethylene Glycol Monostearate	Wax	White	6.6[b]	52	Dispersible	Soluble
Diethylene Glycol Distearate	Wax	Brown	...	48	Insoluble	Soluble
Diethylene Glycol Glyceryl Mono-stearate	Wax	Tan	10.6[b]	−47	Dispersible	Soluble
Ethylene Glycol Monolaurate	Oil	Dark red	8.2	29	Dispersible	Soluble
Ethylene Glycol Mono-oleate	Oil	Dark red	9.5	2	Dispersible	Soluble
Ethylene Glycol Monoricinoleate	Oil	Dark red	...	−45	Insoluble	Partly soluble
Ethylene Glycol Monostearate	Wax	Tan	...	60	Insoluble	Soluble
Ethylene Glycol Distearate	Wax	Brown	...	61	Insolbule	Soluble
Glyceryl Monolaurate	Semisolid	Cream	8.6	24	Dispersible	Soluble
Glyceryl Monomyristate	Wax	Tan	7.6[b]	49	Dispersible	Partly soluble
Glyceryl Mono-oleate	Oil	Orange-red	7.5	5	Dispersible	Soluble
Glyceryl Monopalmitate	Wax	Tan	7.4[b]	54	Dispersible	Partly soluble
Glyceryl Monoricinoleate	Oil	Orange-red	9.3	−50	Dispersible	Insoluble
Glyceryl Monostearate	Wax	Cream	...	56	Insoluble	Soluble
Glyceryl Distearate	Wax	Tan	...	51	Insoluble	Soluble
Glyceryl Tristearate	Wax	White	...	59	Insoluble	Soluble
Mannitol Glyceryl Monolaurate	Paste	Tab	8.3[b]	37	Dispersible	Partly soluble
Mannitol Glyceryl Mono-oleate	Fluid	Yellow	9.5[b]	35	Dispersible	Partly soluble
Mannitol Glyceryl Monostearate	Wax	Tan	9.9[b]	60	Dispersible	Soluble
Polymerized Glycol Monostearate	Soft wax	Cream	2.7	36	Colloidal solution	Insoluble
Propylene Glycol Monolaurate	Oil	Light orange	8.7	14	Dispersible	Soluble
Propylene Glycol Mono-oleate	Oil	Orange	9.6	6	Dispersible	Soluble
Propylene Glycol Monoricinoleate	Oil	Dark amber	...	0	Insoluble	Soluble
Propylene Glycol Monostearate	Wax	Cream	7.1[b]	44	Dispersible	Partly soluble
Propylene Glycol Glyceryl Mono-stearate	Wax	Tan	10.6[b]	64	Dispersible	Soluble
Sorbitol Triricinoleate	Viscous liquid	Brown	...	−40	Insoluble	Soluble
Sorbitol Distearate	Wax	Cream	7.7[b]	50	Dispersible	Soluble
Sorbitol Glyceryl Monostearate	Wax	Tan	9.6[b]	59	Dispersible	Soluble

[a] Courtesy Glyco Products Co., Brooklyn, N. Y.
[b] The *p*H is that of a 5% solution with the exception of those marked (*b*) which are of a 3% solution.

SO_3 content	10%
Moisture content	25%
Softening point	30° C.
*p*H	6.00–6.30

The petrolatum employed possessed the following specifications:

Melting point (Saybolt)	38–43° C.
Specific gravity (60° F.)	0.855–0.860
Viscosity (Saybolt)	58–63
Color (Lovibond)	2 Y (2" cell)
Consistency (A. S. T. M)	170–200

The basic formula, originally prepared with diethylene glycol monostearate, follows:

The glycol ester	16.7 Gm.
Sulfated Hydrogenated Castor Oil	33.3 Gm.
Petrolatum	50.0 Gm.

The constituents were melted, thoroughly mixed, and stirred occasionally while cooling. The data are shown in Table II, column A.

The second formula is based on an ointment base employed by Klauder, Gross and Brown (3). This base originally used glycerin monostearate:

White Wax, U. S. P.	10 Gm.
Hydrous Wool Fat, U. S. P.	5 Gm.
The glycol ester	12.5 Gm.
Stearic Acid, U. S. P.	2.0 Gm.
Petrolatum	75.0 Gm.

The constituents were melted in order of their melting points, thoroughly mixed, and stirred occasionally while cooling. The data are shown in Table II, column B.

The original formula (2) utilized diethylene glycol monostearate and was modified as follows:[2]

The glycol ester	10 Gm.
Sulfated Hydrogenated Castor Oil	20 Gm.
Petrolatum	30 Gm.
Distilled Water	40 Gm.

The first three substances were melted at as low a temperature as convenient and the water, previously heated to the same temperature, was added and agitated on a mechanical mixer. The data are shown in Table II, column C.

TABLE II.—OINTMENT BASES PREPARED WITH GLYCOL ESTERS.

Ester	Nonemulsified A	Nonemulsified B	Emulsified C	Emulsified D	Vanishing E	Vanishing F
"Butyl Cellosolve" Laurate	3½	3	3A	D	D	D
"Buyl Cellosolve" Palmitate	3½	3	3A	D	D	D
"Carbitol" Laurate	3X	3	2A	D	D	D
"Carbitol" Ricinoleate	3	3	2B	D	D	D
"Carbitol" Stearate	3	3	2½A	D	D	D
"Cellosolve" Laurate	3	3½	2½A	D	D	D
"Cellosolve" Ricinoleate	2	...	2A	D	D	D
"Cellosolve" Stearate	3	3	2½A	D	D	D
Diethylene Glycol Monolaurate	3	3	3A†	1A	1C	3A
Diethylene Glycol Dilaurate	3	3	2A	D	D	D
Diethylene Glycol Mono-oleate	3X	3*	2½A†	1A	D	3A†
Diethylene Glycol Glyceryl Mono-oleate	3Y	3	2½A*	1A	2A	3A*
Diethylene Glycol Monopalmitate	3½	3½	3A	3A	3A†	4A
Diethylene Glycol Palmitate Stearate	...	3	...	3A	D	4A
Diethylene Glycol Monoricinoleate "A"	2X	3½	2½A	D	D	D
Diethylene Glycol Monoricinoleate "B"	3½Y	3	2A	D	D	D
Diethylene Gycol Glyceryl Monoricinoleate	4	3	2½A	1A	D	3A
Diethylene Glycol Monostearate	3	3	3A†	3A	4A	4A
Diethylene Glycol Distearate	3Y	4	2B	D	D	D
Diethylene Glycol Glyceryl Monostearate	...	3	...	2C	3A†	4A
Ethylene Glycol Monolaurate	3	D	3	D		
Ethylene Glycol Mono-oleate	3Y	3	D	2A	1B	3A
Ethylene Glycol Monoricinoleate	2Y	2	2½A	D	D	D
Ethylene Glycol Monostearate	3	4*	D	D	D	D
Ethylene Glycol Distearate	3	3†	D	D	D	D
Glyceryl Monolaurate	3	3	2½A*	2½A*	3A	4A
Glyceryl Monomyristate	3	3†	2½A	2½A	3A	4A
Glyceryl Mono-oleate	3	3½	2B	2½A	D	3A
Glyceryl Monopalmitate	3½	3	3A	3A	4A	4A
Glyceryl Monoricinoleate	2	...	2A	2½A	2A	3A
Glyceryl Monostearate	4Y	...	3½A	3A	3A	3½A
Glyceryl Distearate	3	4	2½A	D	D	D
Glyceryl Tristearate	3½Y	4	3A	D	D	D
Mannitol Glyceryl Monolaurate	3	3	2½A	2½A	2A*	3A
Mannitol Glyceryl Monoricinoleate	3	3	...	1A	D	3A
Mannitol Glyceryl Monostearate	3Y*	4*	3A	3A*	3A	3½A*
Polymerized Glycol Monostearate	...	...	..	D	1B	3A
Propylene Glycol Monolaurate	3	2	2B	2A	1A	3A
Propylene Glycol Mono-oleate	2	2	2A	2C	2A	2½A
Propylene Glycol Monoricinoleate	3Y	...	2B*	D	D	D
Propylene Glycol Monostearate	3	3	3A	3A	3A	3½A
Propylene Glycol Glyceryl Monostearate	4	3½	3A*	3A	3A	3½A
Sorbitol Triricinoleate	3½X	3	2A	2B	D	D
Sorbitol Distearate	3½	3½	3A	3A	4A	3A
Sorbitol Monostearate	4	4	3A	3A	3A*	D

Legend: Headings, see text. Consistency: 1—liquid, 2—semisolid, 3—optimum, 4—too firm. Emulsification: A—perfect, B—"creamed," but not broken, C—doubtful, D—broken, *—dark color, †—exceptionally good, X—no separation after 15 hr. at 50° C., Y—slight separation; no designation (column A only) distinct separation.

Emulsified Ointment Bases.—Two basic formulas were used. The liquid petrolatum employed possessed the following specifications:

Specific gravity	0.875–0.885
Color	30 upwards
Viscosity at 100° F. (Saybolt)	200–210
Flash point	360° F.

[2] This product with diethylene glycol monostearate is marketed as *Dermalav*, by Dermal Products Co., Buffalo, N. Y.

The second basic formula was modified from a standard formula containing diethylene glycol monostearate; the resultant product was a soft cream when used with that ester:

The Glycol Ester	14 Gm.
Petrolatum	6 Gm.
Paraffin, U. S. P.	2 Gm.
Liquid Petrolatum	30 Gm.
Distilled Water	48 Gm.

The first four substances were melted in the order of their melting points and agitated in a mechanical mixer with the water, previously heated to the same temperature; excessive heat was avoided. The data are shown in Table II, column D.

Vanishing Ointment Bases.—Two basic formulas were employed. The first is one recommended by Osborne (4) in which glyceryl monostearate is replaced by the glycol ester:

The Glycol Ester	12 Gm.
Glycerin, U. S. P.	3 Gm.
Spermaceti, U. S. P.	3 Gm.
Distilled Water	42 Gm.

The ester and spermaceti were melted and incorporated with the glycerin and water, previously heated to the same temperature. The data are shown in Table II, column E.

The second basic formula is a stearic acid vanishing base usually prepared with diethylene glycol monostearate:

Stearic Acid	13 Gm.
The Glycol Ester	20 Gm.
Water	67 Gm.

The stearic acid and glycol ester were melted and agitated with the water, previously heated. The data are shown in Table II, column F.

SUMMARY

Ointment bases of anhydrous, emulsified and vanishing types were prepared employing forty-five different polyhydric alcohol esters. The following appeared to produce superior ointment bases: diethylene glycol monopalmitate and monostearate; glyceryl monolaurate, monomyristate, monopalmitate, monoricinoleate and monostearate; propylene glycol monostearate; propylene glycol glyceryl monostearate and sorbitol distearate.

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A New System of Color Matching Fluids*

By Kenneth L. Kelly†

Standard color matching fluids were incorporated into the United States Pharmacopœia, Eleventh Revision (1), as a means of evaluating the degree of darkening of solutions resulting from tests for carbonizable substances. Following the pioneering work of Arny, *et al.* (2, 3), three colorimetric solutions were selected as the primary solutions from which these color matching fluids were prepared. The red solution contains cobaltous chloride, the yellow solution contains ferric chloride and the blue solution contains cupric sulfate. The color matching fluids are prepared by combining varying amounts of the three colorimetric solutions with distilled water. In practice, a color matching fluid deeper in color (darker and stronger) than the color of the test solution is selected and the degree of darkening of the test solution is described as being "not deeper than color matching fluid X."

The test for carbonizable substances was developed as a means of limiting the presence of certain adulterants in drugs. It is used, for example, to detect the presence of tartrates in citric acid or the presence of splinters or linters in drugs and chemicals due to faulty preparation or storage, or to limit the amount of oil in paraffin. The test is performed usually by mixing a specific amount of the substance in a definite amount of concentrated sulfuric acid in a test tube, allowing the mixture to stand for fifteen minutes and then comparing the darkening of the sample against a color matching fluid in a similar test tube both viewed transversely against a white background.

The colorimetric solutions are made up in exactly half-normal strength in 2.5 per cent

* Received July 15, 1944, from the AMERICAN PHARMACEUTICAL ASSOCIATION Laboratory, Washington, D. C.

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hydrochloric acid and, when stored in glass-stoppered bottles, are reported to be permanent for a number of years. The color matching fluids, being combinations of these solutions and distilled water, should possess the same permanency provided there is no evaporation or chemical interaction among the constituents. The Pharmacopœia, however, directs that the color matching fluids be discarded after use and not kept until the next day.

ground and the Munsell notation of the color of each fluid was determined according to the method previously published (7, 8). These Munsell notations serve as a permanent record of the color of each matching fluid.

The twenty color matching fluids in the U. S. P. XI are divided into five color groups or series according to their hue, namely, "grayish," "fawn colored," "yellow," "green" and "pink." The matching fluids in each hue series differ from each other in depth. The hues of the matching fluids in any one series are close but there is no regularity in the stepping from matching fluid to matching fluid. Thus, it is not possible to relate the colors of two test solu-

TABLE I.—THE MUNSELL NOTATIONS AND ISCC–NBS COLOR NAMES OF THE MATCHING FLUIDS IN THE U. S. P.[a]

U. S. P. XII Designation	U. S. P. XII Color Group	Munsell Notation		ISCC–NBS Color Name
A	Grayish	7.5Y	9.1/1.7	Faint yellow
B	Grayish	7.5Y	9.07/1.8	Faint yellow
C	Grayish	8.5Y	9.05/2.5	Pale yellow to pale yellow-green
D	Grayish	9Y	8.8/1.8	Pale yellow-green
E	Fawn colored	7Y	8.7/4.7	Pale greenish yellow
F	Fawn colored	5.5Y	9.05/6.3	Light yellow
G	Fawn colored	4.5Y	8.8/6.3	Light yellow
H	Yellow	7.5Y	9.05/6.7	Light greenish yellow
I	Yellow	6.5Y	8.9/9.0	Light to brilliant yellow, light to brilliant greenish yellow
J	Yellow	7.5Y	9.0/10.5	Brilliant greenish yellow
K	Yellow	5Y	8.4/14	Vivid yellow
L	Yellow	3.5Y	8.1/13	Strong yellow to vivid yellow
M	Green	9.5Y	9.05/8.0	Light greenish yellow
N	Green	10Y	9.0/12	Brilliant greenish yellow
O	Green	9Y	8.9/11.5	Brilliant greenish yellow
P	Pink	2Y	8.9/2.3	Very pale orange
Q	Pink	10YR	8.9/2.2	Very pale orange
R	Pink	0.5Y	8.8/2.3	Very pale orange
S	Pink	8R	9.0/2.8	Pale pink
T	Pink	10YR	8.6/2.4	Very pale orange

[a] Determined for a 2-cm. depth.

EXPERIMENTAL

In order to determine the permanency of the color matching fluids, the past day's set of matching fluids was retained until the succeeding day's set was prepared and then the two were compared under the most favorable conditions of lighting and viewing. Between matching fluids several days old no apparent change was noticeable, but evaporation will take place slowly, and even when the tubes are tightly corked, slight differences in color in several of the tubes will be noticeable after a week.

As a means of studying the spacing or stepping (difference in depth between adjacent matching fluids) of the individual matching fluids and their permanence, the Munsell notations (4) were determined by one observer for the colors of several sets of these color matching fluids and the results averaged. These notations were determined in a flat-bottomed clear glass vial whose internal diameter was 17 mm. and at a 2-cm. depth. The vials were viewed against a white background illuminated with ICI illuminant "C" (5, 6) equivalent to standard daylight such as that obtained at an unobstructed north window from a slightly overcast sky. The 2-cm. depth was chosen because it duplicated very closely the colors obtained in the test tubes in the test for carbonizable substances. A white, two-aperture, color matching shield was used as the background and the Munsell notation of the color of each fluid was determined according to the method previously published (7, 8). These Munsell notations serve as a permanent record of the color of each matching fluid.

The twenty color matching fluids in the U. S. P. XI are divided into five color groups or series according to their hue, namely, "grayish," "fawn colored," "yellow," "green" and "pink." The matching fluids in each hue series differ from each other in depth. The hues of the matching fluids in any one series are close but there is no regularity in the stepping from matching fluid to matching fluid. Thus, it is not possible to relate the colors of two test solutions by a comparison of the appropriate matching fluids in this system. The Munsell notations of the colors of the twenty matching fluids with their corresponding ISCC–NBS color names are given in Table I.

Since the U. S. P. color matching fluids are not evenly stepped, an attempt was made to develop a new series of matching fluids based on recognized colorimetric principles. In the ideal color matching fluids, the hue of each series should be constant and the stepping between successive matching fluids in any series should be equal. The stepping should be the same for the matching fluids in each series.

Four evenly spaced hues were chosen to cover the hue range observed in tests for carbonizable substances; these hues are orange, yellowish orange, yellow and greenish yellow. For each of these four hues, there will be a series of matching fluids decreasing in depth (becoming paler) from the deepest to the palest in each series. Two other series were developed, a weak orange and a weak yellow, to take the place of the "grayish" and the "pink" color series in the Pharmacopœia. In order to simplify the preparation of the matching fluids, specific amounts of the three colorimetric solutions and distilled water are combined to form the deepest matching fluid in each series, called here the "master fluid."

By combining the three colorimetric solutions in varying proportions with distilled water, six master

fluids were prepared so that their colors matched, within experimental error, the colors previously selected. Specific dilutions of each master fluid were then made to form the other matching fluids in that series, the dilutions being so chosen that the and greenish yellow master fluids were planned to fall in the centers of the orange, yellowish orange, yellow and greenish yellow color-name segments (8). Their hues are 5YR, 9.5YR, 4.5Y and 9.5Y, all within one-half hue step of the desired hue. The

TABLE II.—THE COMPOSITIONS, MUNSELL NOTATIONS AND ISCC–NBS COLOR NAMES OF THE PROPOSED COLOR MATCHING FLUIDS

Fluid Designation	Composition of Matching Fluid, Cc. Master Fluid	Dist. Water	Munsell Notation		ISCC–NBS Color Name	Composition of Master Fluid, Cc. Co[a]	Fe[b]	Cu[c]	Dist. Water
				(*a*)	Orange series	2.6	2.2	0.2	...
O_0	...	5.0		10/	Colorless				
O_1	0.3	4.7	6.5YR	9.0/1.7	Very pale orange				
O_2	0.7	4.3	6.5YR	8.8/3.3	Pale orange				
O_3	1.2	3.8	6YR	8.3/5.3	Pale orange				
O_4	1.8	3.2	5.5YR	7.9/7.3	Light orange				
O_5	2.7	2.3	5.5YR	7.7/9.3	Light orange				
O_6	3.8	1.2	5YR	7.3/11.5	Strong orange				
O_7	5.0	...	5YR	6.6/14	Strong orange				
				(*b*)	Weak orange series	0.5	0.4	0.4	3.7
wO_0	...	5.0		10/	Colorless				
wO_1	2.1	2.9	6YR	8.9/0.8	Faint pink				
wO_2	3.8	1.2	6.5YR	8.8/1.7	Very pale orange				
wO_3	5.0	...	7.5YR	8.6/2.2	Very pale orange				
				(*c*)	Yellowish orange series	1.6	3.2	0.2	...
yO_0	...	5.0		10/	Colorless				
yO_1	1.0	4.0	2Y	8.6/4.0	Pale yellowish orange				
yO_2	1.8	3.2	1.5Y	8.3/6.3	Weak yellowish orange				
yO_3	2.7	2.3	1Y	7.9/8.5	Moderate yellowish orange				
yO_4	3.8	1.2	10YR	7.6/10.5	Moderate yellowish orange				
yO_5	5.0	...	9.5YR	7.3/12.5	Strong yellowish orange				
				(*d*)	Yellow series	0.8	4.0	0.2	...
Y_0	...	5.0		10/	Colorless				
Y_1	0.9	4.1	7.5Y	8.95/3.3	Pale greenish yellow				
Y_2	1.6	3.4	6.5Y	8.8/6.0	Light yellow to light greenish yellow				
Y_3	2.7	2.3	5.5Y	8.6/8.5	Light yellow				
Y_4	3.9	1.1	5Y	8.2/10.7	Strong yellow				
Y_5	5.0	...	4.5Y	7.8/12.5	Strong yellow				
				(*e*)	Weak yellow series	0.9	1.4	0.8	6.9
wY_0	...	5.0		10/	Colorless				
wY_1	2.1	2.9	5Y	9.0/0.8	Faint yellow				
wY_2	3.6	1.4	5Y	8.9/1.8	Faint yellow				
wY_3	5.0	...	5Y	8.7/2.3	Pale yellow				
				(*f*)	Greenish yellow series	0.2	4.6	0.2	...
gY_0	...	5.0		10/	Colorless				
gY_1	0.7	4.3	9.5Y	9.0/3.3	Pale greenish yellow				
gY_2	1.5	3.5	9.5Y	8.9/5.5	Light greenish yellow				
gY_3	2.4	2.6	9.5Y	8.9/7.5	Light greenish yellow				
gY_4	3.6	1.4	9.5Y	8.7/10	Brilliant greenish yellow				
gY_5	5.0	...	9.5Y	8.3/12	Strong greenish yellow				

[a] Co—Cobalt Chloride C. S., U. S. P.
[b] Fe—Ferric Chloride C. S., U. S. P.
[c] Cu—Cupric Sulfate C. S., U. S. P.

stepping between successive matching fluids from the deepest, the master fluid, to the palest, distilled water, was equal. The colors of all of these matching fluids should be subjected to spectrophotometric analysis and calculation (9, 10) but for practical purposes and for the use to which they will be put in these tests, the accuracy of the Munsell notations will be sufficient.

The hues of the orange, yellowish orange, yellow hue of the weak orange master fluid, 7.5YR, was selected to fall midway between the orange and yellowish orange master fluids whose hues are 5YR and 9.5YR. Likewise the hue of the weak yellow master fluid, 5Y, was selected to fall between the yellow and the greenish yellow master fluids whose hues are 4.5Y and 9.5Y. A small amount of blue colorimetric solution is added to each master fluid to darken its color.

TABLE III.—THE RELATIVE DEPTH OF THE U. S. P. AND THE PROPOSED COLOR MATCHING FLUIDS

Proposed Color Matching Fluids			U. S. P. Color Matching Fluids	
Matching Fluid	Munsell Notation		Matching Fluid	Munsell Notation
O_0	10/			
O_1	6.5YR	9.0/1.7		
			S	8R 9.0/2.8
O_2	6.5YR	8.8/3.3		
O_3	6YR	8.3/5.3		
O_4	5.5YR	7.9/7.3		
O_5	5.5YR	7.7/9.3		
O_6	5YR	7.3/11.5		
O_7	5YR	6.6/14		
wO_0	10/			
wO_1	6YR	8.9/0.8		
wO_2	6.5YR	8.8/1.7		
			Q	10YR 8.9/2.2
			R	0.5Y 8.8/2.3
wO_3	7.5YR	8.6/2.2	T	10YR 8.6/2.4
yO_0	10/			
yO_1	2Y	8.6/4.0		
			G	4.5Y 8.8/6.3
yO_2	1.5Y	8.3/6.3	G	4.5Y 8.8/6.3
yO_3	1Y	7.9/8.5		
yO_4	10YR	7.6/10.5		
yO_5	9.5YR	7.3/12.5		
Y_0	10/			
Y_1	7.5Y	8.95/3.3		
			E	7Y 8.7/4.7
Y_2	6.5Y	8.8/6.0		
			F	5.5Y 9.05/6.7
			H	7.5Y 9.05/6.3
Y_3	5.5Y	8.6/8.5	I	6.5Y 8.9/9.0
			J	7.5Y 9.0/10.5
Y_4	5Y	8.2/10.7		
Y_5	4.5Y	7.8/12.5	K	5Y 8.4/14
Y_6	4.5Y	7.8/12.5	L	3.5Y 8.1/13
wY_0	10/			
wY_1	5Y	9.0/0.8		
			A	7.5Y 9.1/1.7
			B	7.5Y 9.07/1.8
wY_2	5Y	8.9/1.8	D	9Y 8.8/1.8
			P	2Y 8.9/2.3
wY_3	5Y	8.7/2.3		
gY_0	10/			
			C	8.5Y 9.05/2.5
gY_1	9.5Y	9.0/3.3		
gY_2	9.5Y	8.9/5.5		
			M	9.5Y 9.05/8.0
gY_3	9.5Y	8.9/7.5		
gY_4	9.5Y	8.7/10		
			N	10Y 9.0/12
			O	9Y 8.9/11.5
gY_5	9.5Y	8.3/12		

Table II shows the compositions of the master fluids, the compositions of the diluted matching fluids in each series, and the Munsell notation of each matching fluid with its corresponding ISCC–NBS color name. Each matching fluid is given a two-part designation, the first part (0, w0, y0, Y, wY or gY)[1] indicating the series to which it belongs, the second part a serial number (1 to 7) for position in the series. Distilled water is designated simply as zero (0).

[1] The designations given the color matching fluids are recognized abbreviations used in the ISCC–NBS system of color names; 0 for orange, wO for weak orange, yO for yellowish orange, Y for yellow, wY for weak yellow, and gY for greenish yellow.

Upon the completion of the proposed matching fluids, it was then possible to determine the correlation between the two systems. Each of the U. S. P. matching fluids was compared with the proposed matching fluids and its relative hue and depth were noted. This relationship is shown in Table III. When a U. S. P. matching fluid exactly matches a proposed matching fluid in depth it is placed opposite its counterpart, otherwise placing a U. S. P. matching fluid between two proposed matching fluids indicates that it is intermediate in depth. From Table III it will be seen that the judgment of depth is not one of lightness (Munsell value) only, but a combination of lightness and saturation or strength (Munsell chroma). In the ISCC–NBS system of color names, deep means dark and strong

so therefore depth means a combination of darkness and saturation.

The permanency and rules governing the use of the proposed matching fluids are the same as for the U. S. P. matching fluids since the same colorimetric solutions are used. However, no mention is made in the present Pharmacopœia of the source of illumination to be used. Since illuminant "C," light obtained from an unobstructed north window from a slightly overcast sky, or its equivalent such as a Macbeth daylight lamp or daylight fluorescent unit, is to be preferred in scientific color discriminations of this type, it is recommended that illuminant "C" be prescribed by the Pharmacopœia.

SUMMARY

1. The Munsell notations and ISCC–NBS color names of the U. S. P. color matching fluids have been determined.

2. It was observed that the U. S. P. color matching fluids were not evenly stepped in depth of color.

3. Six new series of color matching fluids are proposed which are uniform in stepping and which cover evenly the range of color resulting from carbonization tests.

4. The Munsell notations and ISCC–NBS color names have been determined for the proposed color matching fluids.

5. The proposed color matching fluids are compared with the U. S. P. color matching fluids.

6. A standard source of illumination (illuminant "C," equivalent to standard daylight) is recommended for use with the color matching fluids.

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Book Reviews

Biological Symposia. X Frontiers in Cytochemistry, edited by Normand L. Hoerr. The Jaques Cattell Press, Lancaster, 1943. 334 pp. 24.5 x 17 cm. Price, $3.50.

This publication is based upon a symposium in honor of Professor Bensley held at the University of Chicago in 1942. Such prominent cytologists and biochemists as E. S. G. Barron, E. V. Cowdry, N. L. Hoerr and K. G. Stern took part formally in the symposium. The following topics were discussed: the chemical structure of cytoplasm as investigated in Professor Bensley's laboratory during the past ten years, some considerations on the application of biological oxidation-reduction reaction systems to the study of cellular respiration, ultracentrifugal studies on cytoplasmic components and inclusions, electrolytic solutions compatible with the maintenance of protoplasmic structures, distribution of nucleic acids in the cell and morphological constitution of cytoplasm, experimental epidermal methylcholanthrene carcinogenesis in mice, histochemical analysis of changes in rhesus motor-neurons after root section, methods of isolation of morphological constituents of the liver cell, electrolytes in the cytoplasm, mineral distribution in the cytoplasm, studies on the macromolecular particles endowed with specific biological activity, and the chemistry of cytoplasm.

The topics are so diversified that any review would be inadequate. Suffice it to say that chemists who are cytologically minded and cytologists who are chemically oriented will find this symposium stimulating, for it presents the hybrid point of view. To read some of these excellent discussions is to realize that cytology has left a static age for a new and dynamic viewpoint.—M. W. GREEN.

The Chemical Formulary, Volume VI, by H. Bennett. Chemical Publishing Company, Brooklyn, 1943. xx + 635 pp., 13.5 x 21.5 cm. Price, $6.00.

This latest volume of the popular *Chemical Formulary* differs little from its predecessors. A number of wartime features will be appealing to some. For example, a complete tabulation of war gases, pro-

tective agents and antidotes are included. Of practical importance is the inclusion of a table of possible substitutes for scarce substances.

In the drug section, the presence of preparations containing sulfa drugs is evidence of up-to-dateness. This section, however, suffers from its old ailment of impracticality and failure to take legal requirements into account.—M. W. GREEN.

Clinical Diagnosis by Laboratory Examinations, by JOHN A. KOLMER. D. Appleton–Century Company, New York, 1943. 1239 pp., 75 illus. Price, $8.00.

This book, which is a companion to *Approved Laboratory Techniques* by Kolmer and Boerner, is noteworthy for its text and scope. The size of the book speaks for its completeness and the inclusion of discussions of such relatively new material as the "Rh factor" is evidence of its timeliness.

The book is divided into three parts. Part I deals with the clinical interpretation of laboratory examinations, the evaluation of which requires a knowledge of normal values as well as the physiological mechanisms and functions concerned with their variation in health and disease and six hundred forty-one pages are devoted to a very able and thorough discussion of these factors. The tabular summaries in this part as well as in Part II are of great value.

Part II is devoted to the practical application of laboratory examinations in the clinical diagnosis of disease. Here is found the description of diseases of the various systems of the body and the discussion of the salient etiology, pathology and clinical features, the emphasis being placed on diagnosis by laboratory methods. The consideration given to falsely positive reactions is helpful and the introduction of colored plates to illustrate intradermal tests is highly instructive.

Part III is devoted to technical procedures and deals with methods for performing the more important and frequently used laboratory examinations. This portion of the book, valuable as it is, is not as exhaustive a treatment as is to be found in the author's *Approved Laboratory Techniques*.

This book will be found useful as a reference to those persons in the pharmaceutical field who are interested in clinical laboratory practice and who desire to have interpretive information available to their physician clients.—HAROLD V. CONNERTY.

Emulsion Technology, edited by H. BENNETT. Chemical Publishing Company, Brooklyn, 1943. xi + 290 pp. 22 x 13.5 cm. Price, $5.00.

Many treatises on emulsion technology have been published. This book is a symposium sponsored by the Leather Trades Chemists (English), and is unique in its balance between theory and practice. The individual chapters were written by different people acknowledged to be experts in their fields.

Outstanding is the chapter on the fundamental principles of practical emulsion manufacture by Miss R. M. K. Cobb. Workers in the emulsion field will appreciate her statement regarding the potential energy of an emulsion, that "stored in a tank, it has nothing else to do with that energy all day and night but coalesce, cream and break into its original components." Her discussion of emulsion quality in terms of Stokes' equation is most enlightening. The discussion of the role of the emulsifying agent is also helpful.

The chapter by Walsh and Frazer on the use of highly dispersed emulsions in the treatment of toxemic conditions presents some novel concepts which should stimulate further research although in the opinion of the reviewer, the clinical application is doubtful. Clayton's review of the patent literature is especially useful to manufacturers.

Other phases of emulsion technology are too numerous to mention. The design of emulsion machines, industrial emulsion formulation, agricultural sprays and food emulsions are only a few of the topics reviewed in this handy little book.—MELVIN W. GREEN.

Organic Chemistry, by LOUIS F. FIESER and MARY FIESER. D. C. Heath Company, Boston, 1944. xii + 1091 pp. 23.5 x 15 cm. Price, Trade Edition, $8.00; College Edition, $6.00.

With thousands upon thousands of organic compounds now catalogued and hundreds of organic reactions known, to present a scholarly text which is at the same time readable is difficult. The Fiesers have solved this problem in their new text admirably.

A glance at advanced texts will show one of two basic fallacies Either modern theory is sacrificed to make room for a hodgepodge of organic compounds and reactions (often poorly indexed) or "old-fashioned" organic chemistry is made to play second fiddle in a symphony of ponderous theory. Neither of these types of books fulfills the needs of the student or the research worker very effectively.

The more theoretical phases of organic chemistry are presented in an interesting fashion by the Fiesers. In the earlier portions of the book, the student is asked to rely upon empirical relationships with little questioning. Later, these relationships are developed in terms of electronic balance, resonance, steric properties and other modern concepts, the development being so gradual that the student is at no time inundated with foreign concepts. At the same time there is never a feeling that one is being fed a diet of soft foods.

The reviewer believes that organic chemistry should be taught visually, even at the graduate level. This has been done here, for nearly all reactions are graphically illustrated.

Several essay chapters are included on the applications of organic chemistry to technological fields such as the petroleum and rubber industries as well as biological and medical applications of organic chemistry. The timeliness of these sections is shown by the inclusion of the Baruch report on synthetic rubber.

The section on chemotherapy, disappointing as all such sections must be when incorporated into general texts, suffers from brevity but makes up for its curtness by its meatiness. The section can be read gainfully by students of medicinal chemistry, and it most certainly cannot fail to stimulate the student of general organic chemistry to see that this is where organic chemistry reaches its highest goal—the alleviation of human suffering.

When one appreciates that the hectic paces of war required the senior author to write much of the text in "trains, planes, hotel rooms and army camps," one is doubly appreciative of the fine quality of the writing. This book should occupy a prominent place in the library of the pharmaceutical organic chemist.—M. W. GREEN.